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CHARACTERIZATION OF ESCHERICHIA COLI RELATED TO CONSTRUCTION SITE SEDIMENT BASINS

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CHARACTERIZATION OF *ESCHERICHIA COLI* RELATED TO CONSTRUCTION SITE SEDIMENT BASINS

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by
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ABSTRACT

Large construction sites can have significant temporary and permanent impacts on the physical landscape. Excess sediment is frequently deposited into nearby surface waters, altering benthic habitat, reducing water clarity and transporting other pollutants such as enteric bacteria. To capture eroded soil and attenuate storm discharge peaks, many permitted construction projects larger than 10 acres in South Carolina require the installation of a sediment detention basin. Sediment-laden runoff is routed to a newly excavated impoundment, where larger particles settle out of suspension. Thus an entirely new hydrologic feature designed to concentrate eroded sediment and water is introduced into the landscape.

Eight construction projects and their associated sediment basins were monitored in Anderson, SC during the spring, summer and autumn of 2008 to evaluate *Escherichia coli* (*E. coli*) densities and assess relationships with observed environmental variables. Dry and wet-weather samples were collected from basin inlets, outlets, water column and deposited sediments. Bacterial concentrations from construction site runoff measured at inlets (mean = 771 MPN/100 ml) were consistently and significantly higher than water quality criteria established for primary contact recreation by the US Environmental Protection Agency. Basin discharge measured at outlets showed significantly higher bacterial concentrations (mean = 1368 MPN/ 100 ml; t-stat = 3.54; p = .0036)

than those found in construction site runoff and also exceeded EPA standards. Within sediment basins, both mean water column (877 MPN/100 ml) and mean sediment (1.8E+5 MPN/100 ml) *E. coli* densities were higher than recommended EPA criteria, with mean concentrations in the sediments significantly exceeding the corresponding overlying water column (t-stat = 5.51; $p < .0001$). Aggregated data suggest these sediment control practices are not acting to reduce bacterial concentrations, but rather appear to be serving as reservoirs for viable *E. coli* and net sources of bacterial loading to receiving waters.

Quantification of construction site bacterial runoff, potential basin-related bacterial contamination, and examination of site discharges will assist stormwater regulators and engineers in evaluating the efficacy of state construction permit standards and confirm whether site design practices are protective enough of receiving water quality. Results may also provide information to assess whether the present course of construction-related stormwater design and management is suitable or sustainable.

To address future publication considerations, this dissertation is divided into 5 discrete sections. The initial chapter addresses supporting research, detailed site descriptions, materials and methods, and consequent cited literature. The subsequent 2 sections were written and formatted specifically for submission to identified peer-reviewed scientific journals. Chapter 2 (Journal of the American Water Resources Association) quantifies mean *E. coli* densities across all monitored basins and sample locations and evaluates these data with respect to water quality criteria established by EPA. Chapter 3 (Journal of Applied and Environmental Microbiology) utilizes bivariate correlation and multiple regression to evaluate *E. coli* densities and assess their relationship to observed physical and chemical conditions. One monitored site was determined to have significant design and hydrologic differences and was therefore isolated and examined separately in Chapter 4. Comprehensive summary conclusions for this research are consolidated and provided in Chapter 5.

DEDICATION

This dissertation is dedicated to my parents, George and Susanne Sawyer. The boundless support and encouragement they consistently provide created the foundation necessary to complete such a meaningful endeavor. Their own writing, whether exhibited through purposeful correspondence or a simple book jacket inscription, is evidence of their own abundant spiritual and intellectual curiosity. I will forever be indebted to them for any achievements I might enjoy as a scholar, educator, husband and father.

ACKNOWLEDGMENTS

There are several individuals who deserve to be recognized for their contributions to this research project. City of Anderson engineer Don Chamblee, was instrumental in obtaining permission to access monitored sites. Jeremy Pike assisted in numerous aspects of field research as well as analysis. Clemson Extension collaborators Dave Joyner and Katie Giacalone provided important spatial support. My friend and colleague Patrick Jodice served as sounding board and statistical reality check. Patricia Layton, Chair of the Department of Forestry and Natural Resources at Clemson, provided space (affectionately called the dungeon) in the basement of Lehotsky Hall which allowed me to complete the analysis and writing portion of this effort in relative peace.

I'd also like to acknowledge and thank my family, each of whom has contributed heroically to these efforts. Hannah and Ben did not ask to spend weekends or evenings with me in the field or waiting outside the lab, but they endured each instance with great curiosity and much-appreciated humor.

Finally, exceptional gratitude is extended to my wife Karen, who has been aboard for this voyage largely as a conscripted navigator. She's been an able lab assistant, cornerstone family rock, and unlicensed psychotherapist – each function essential, and I love her very much.

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CHAPTER ONE

INTRODUCTION

Each year thousands of acres of land undergo construction-related land disturbance in the rapidly developing Piedmont region of the southeastern United States. Mobilized sediment contained in site runoff is routinely deposited within creeks, rivers, lakes, and other nearby surface waters, adversely affecting the habitat of important aquatic species, reducing water clarity and transporting other potentially harmful pollutants such as adsorbed *Escherichia coli* (Wood and Armitage, 1997). Determining relationships between the presence, transport and fate of sediment-associated bacteria is of primary concern in South Carolina as the 2008 Section 303(d) list of impaired waterbodies indicates more contamination from fecal coliform than any other single pollutant (SC DHEC, 2008).

Bacteriological water quality criteria have existed for decades within the United States as well as other developed nations. Such standards are largely based on concentration estimates of designated indicator species correlated with gastrointestinal illness rates in paired beach swimming studies (US EPA, 1986). Occurrence of food- and water-borne illness related to certain specific pathogens has measurable economic impacts associated with medical costs and decreases in job productivity. In 2007 alone, an estimated $460M was lost resulting from a single strain of shiga toxin-producing *E. coli* (Frenzen, 2007). More recent comprehensive reviews of research conducted since criteria were first established in 1986 confirm that published results continue to support the use of *enterococci* and *E. coli* as useful predictors of epidemiological health for recreational waters (Wade *et al*., 2003). Establishing or maintaining robust monitoring protocols is essential to protecting public health and economic well-being.

Bacteria are principle components of naturally occurring carbon and nutrient cycling in the environment. Genotypic and phenotypic diversity allows these organisms to survive under a broad range of physical, chemical and biological conditions (Winfield *et al.*, 2003; Maier *et al.*, 2000). Ishii and Sadowsky (2008) suggest the ability of certain enteric bacterial species like *E. coli* to survive long-term outside a host environment is likely due to their ability to acquire energy by various means. In essence, *E. coli* can become "naturalized" into the broader microbial community because it can exist under aerobic and anaerobic conditions, survive in a variety of temperatures, while needing only simple nutrients and trace elements to grow (Davis *et al.*, 2005; Byappanahalli *et al.*, 2003; Gagliardi *et al.*, 2002; An *et al.*, 2002).

A developing body of research has shown that if established in the natural environment, *E. coli* can persist throughout the year, serving as a continuous bacterial source (Whitman *et al*., 2006; Byappanahalli *et al*., 2003; Gagliardi *et al.*, 2002). Because a significant fraction of bacteria are associated with soil, runoff laden with newly eroded and suspended sediment can serve as a secondary source of higher *E. coli* concentrations to receiving-waterbodies (Wu *et al.*, 2009; Jamieson *et al*., 2005). Characklis *et al*. (2005) found microbial adsorption varies by microorganism, with 20-30% of viable *E. coli* showing consistent affinity for settleable particle sizes.

Once mobilized, the fate of sediment-associated bacteria is determined in large measure by site-specific hydrologic conditions. Regional studies have found significant correlation between elevated sediment loads and correspondingly high concentrations of fecal coliform bacteria in Piedmont stream systems (Jolley, 2005). Jolley determined indicator bacteria and other waterborne pathogens adsorbed to sediment particles survive following deposition and further, that bacteria existing within this substrate environment can be resuspended and transported following perturbation. Certain pathogenic bacteria in bottom sediments have been found to survive significantly longer than populations found in overlying water columns (Burton *et al*., 1987).

There is a long-established link between sediment and correspondingly high levels of bacteria in lentic systems. Bottom sediments have been shown to act as reservoirs of indicator bacteria and other waterborne pathogens (Davies and Bavor, 2000; Howell *et al*., 1996; LaLiberte and Grimes, 1981). Davis *et al*. (2005) concluded that pond sediments can sustain viable populations of *E. coli* for several months with no external input and that these bacteria may be resuspended back into the water column by turbulent flow associated with storm conditions.

Specifically, construction site sediment basins have been shown to raise ambient stream total suspended solids (TSS) in receiving waters under storm conditions (Ehrhart *et al*., 2002). Ehrhart demonstrated that preferential settling within the basin of larger eroded particles produced effluent containing a higher proportion of finer suspended sediments downstream, as measured by particle size distribution. While certain practices such as baffles or skimmers can further reduce suspended material in basin discharge (Thaxton and McLaughlin, 2004), some fraction of sediment, both newly eroded and resuspended, will be contained within the effluent. Controlled research over 8 years conducted in experimental sediment basins found that on average, 24% of sediment lost through discharge represented resuspension of previously deposited bottom sediments (Jarrett, 2001; Fennessey and Jarrett, 1996).

Beyond trapping efficiency, hydrodynamic modeling and evaluation of sediment-specific discharge impacts on downstream biota, construction site sediment basins have not been the subject of ecologically focused research. To address state regulations, construction site sediment ponds are engineered in South Carolina to capture a minimum 80% of TSS in order to meet the settleable solids criteria of the Stormwater Standards and Sediment Reduction Regulation (SCDHEC, 2002).

Given the association between eroded soils, suspended sediments, bottom sediments and the ubiquitous nature of enteric bacteria in natural ecosystems, research was needed on excavated basins used to control sediment from construction sites. The purpose of this research was to evaluate *E. coli* densities in construction-derived runoff in the Piedmont of South Carolina; assess whether these basin systems created for controlling sediment and stormwater in the region are acting as sources, sinks or reservoirs for potential pathogens; and examine relationships between these observed bacterial concentrations and corresponding environmental variables.

SITE DESCRIPTIONS

Data were collected from 8 construction site sediment basins associated with permitted land disturbance activities in Anderson, South Carolina (Figure 1.1). Located in the Piedmont physiographic province, site soils are characterized primarily by the Cecil series, which is a moderately to well-drained clay loam having dominantly clay subsoil and a clay content ranging between 5-35% (USDA-SCS, 1993). Mean annual precipitation is 127 cm and mean annual temperature is 16.3℃. More detailed site descriptions and representative photographs are provided in subsequent sections. Designed surface area and storage for all basins are given for the 2-year storm elevation.

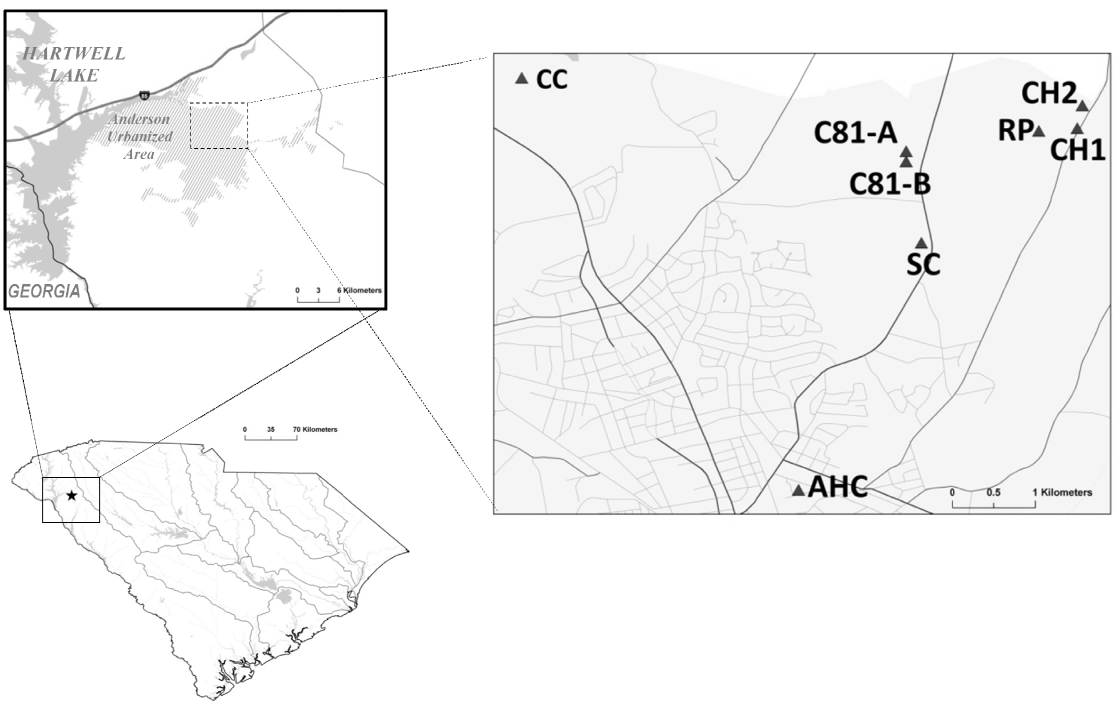

Figure 1.1 - Location of study area. Eight construction site sediment basins were dry- and wet-weather sampled from March-October of 2008. Site designations are shown on right.

AHC Sediment Basin

The AHC site (Figure 1.2) drains 2.6 hectares, has a surface area of 1,230 m^2 and a storage capacity for the 10-year, 24-hour storm event of 925 m^3. Native site soils were of the Cecil series, and the previous land use was mixed-use industrial. Because excavation of this basin was completed following onset of data collection, each of the first 6 contributing storm events was sampled. The site was hydro-seeded during the course of sampling, which established a dense grass cover on basin slopes and even into bottom areas subject to inundation following rainfall.

Figure 1.2 - AHC Sediment Basin. On left, collection of water column composite sample. On right, basin showing box outlet riser and inlet pipe with headwall following initial storm event.

CC Sediment Basin

The CC site (Figure 1.3) drains 19.4 hectares, has a surface area of 2,091 m^2 and a storage capacity for the 10-year, 24-hour storm event of 8,737 m^3, making it the largest basin sampled. Native soils were of the Cecil series and the previous land use was mixed-use industrial. The nature of the permanent pool and consistent discharge from the CC site allowed samples to be collected each week during the course of the research project in addition to any dates involving rainfall. CC was the oldest operating sediment basin included in this project, draining active land-disturbance and highly exposed soil for 14 months prior to the onset of sampling. Time of concentration was less than 15 minutes and consequently no inlet samples were collected as part of this study.

Figure 1.3 - CC Sediment Basin. On left, outlet riser pipe with visible perforations allowing continuous discharge. On right, track hoe is visible on basin bank.

C81-A Sediment Basin

The C81-A site (Figure 1.4) drains 4.6 hectares, has a surface area of 1,280 m^2 and 10-year, 24-hour storm event storage of 1,157 m^3. Native site soils are of the Cecil-Hiwassee association, and the previous land use was pasture/forest. The basin inlet pipe, which can be seen on the left in the background of Figure 1.4, had its invert below deposited sediment. This blockage affected flow from the pipe and prohibited the possibility of collecting samples not compromised by pond backflow.

Figure 1.4 - C81-A Sediment Basin. On left, composite sediment sample being collected with drill pump. On right, reinforced concrete pipe outlet riser.

C81-B Sediment Basin

The C81-B site (Figure 1.5) drains 1.3 hectares, has a surface area of 237 m^2 and 10-year, 24-hour storm storage capacity of only 352 m^3, making it the smallest basin sampled Its previous land use and native soils match those for C81-A. Erosion control matting was installed at C81-B to stabilize steep banks. Rooted grasses were also established within much of the area around the basin bottom. A parking area was added to the C81-B catchment during the fifth month of sampling.

Figure 1.5 - C81-B Sediment Basin. On left, basin slopes show erosion control mats. On right, corrugated metal outlet riser for this small basin.

CH1 Sediment Basin

The CH1 site (Figure 1.6) drains 2.3 hectares, has a surface area of 1,086 m^2 and a 10-year, 24-hour storm storage capacity of 751 m^3. The native soil was Cecil series and the previous land use was forested. Primary basin de-watering for the CH-1 site was through a small diameter polyvinyl chloride (PVC) riser pipe with a 2 cm orifice configuration. Outlet samples were collected at the discharge point of the PVC pipe.

Figure 1.6 - CH1 Sediment Basin. On left, basin is shown following required maintenance-related excavation. On right, PVC pipe outlet riser.

CH2 Sediment Basin

The CH2 site (Figure 1.7) drains 2.7 hectares, has a surface area of 948 m^2 and a 10-year, 24-hour storm storage capacity of 729 m^3. The native soil was Cecil series and the previous land use was forested. CH1 and CH2 were both constructed for a development of detached duplexes. Approximately 25% of the site had dwellings, while the remainder was undergoing active construction. At the onset of sampling, both CH1 and CH2 required significant maintenance. Accumulated sediment in the basins prohibited any sample collection since runoff was never impounded. Following required re-excavation of the basins, samples were collected during subsequent storm events.

Figure 1.7 - CH2 Sediment Basin. On left, prior to required maintenance, a channel was dug by hand to address occluded inlet pipes. On right, the CH2 basin following excavated maintenance.1

RP Sediment Basin

The RP site (Figure 1.8) drains 7.1 hectares, has a surface area of 1,062 m^2 and a 10-year, 24-hour storm storage capacity of 1,477 m^3. Native soils were of the Cecil-Hiwassee association and the previous land use was pasture. RP discharged to a roadside ditch immediately below the site. The RP site was characterized by residential construction taking place during the entire period of sampling. By the outset of basin monitoring, roads and drainage had already been installed. However, the greatest proportion of the site remained graded and largely non-vegetated. Deer tracks and feces were present on numerous sampling dates within the area subject to inundation by stormwater.

Figure 1.8 - RP Sediment Basin. On left, typical reinforced concrete inlet pipe with rip-rap apron. On right, deer tracks within basin perimeter, which is surrounded by 6-ft chain link fence.

SC Sediment Basin

The SC site (Figure 1.9) drains 1.4 hectares, has a surface area of 836 m^2 and a 10-year, 24-hour storm storage capacity of 782 m^3. Native site soils were of the Cecil series and land use was listed in the site plans as 'previously graded'. SC was the oldest pond sampled, with pond excavation being completed 15 months prior to the inception of project sampling. The SC site also included an inlet pipe that was occluded during a majority of sampled storms. Toward the end of the research project, an additional inlet pipe was installed, draining from a re-graded section of the initial disturbed area. An opportunity to collect samples from the new inlet pipe did not present itself.

Figure 1.9 - SC Sediment Basin. On left, this basin was characterized by emergent vascular vegetation established at basin perimeter. On right, new inlet pipe being installed.

MATERIALS AND METHODS

To evaluate changes in bacterial density over time within basins, the frequency for sampling was established to be every 7 calendar days in addition to any rain event which produced runoff. Since the time of concentration for these relatively small catchments was minimal, it was important to be on site to collect grab samples whenever a runoff event occurred. In order to evaluate predictive values using bivariate correlation and multiple regression analysis, 8 physical, chemical and biological parameters were measured. Temperature and pH were measured using a Beckman 255 dual parameter probe (Beckman-Coulter, 2008). Dissolved oxygen (DO) was measured using a YSI-55 handheld probe and conductivity was determined by a YSI-30 meter (YSI, 2004). Rainfall depth was evaluated during each site visit using a Chaney Instruments 840 rain gauge. Days since last rainfall (DSLR) was determined by arithmetic computation based on the most immediate previous date of observed rainfall.

For each sediment basin, there were 4 possible sample locations (Figure 1.10). During runoff events, 500 ml samples were collected at the basin inlet pipe, which led from the active construction site into the detention pond (Inlet). Whenever discharge was occurring from the basin, effluent samples were also collected (Outlet). Water column (WC) samples were obtained using a composite of 500 ml samples collected at representative locations (near outlets, inlets, and perimeter) using a long-handled polyethylene dipper and conveyed into an autoclavable 20 liter carboy. Contents were shaken vigorously for 60 seconds and a single 500 ml sub-sample was drawn into a sterile bottle.

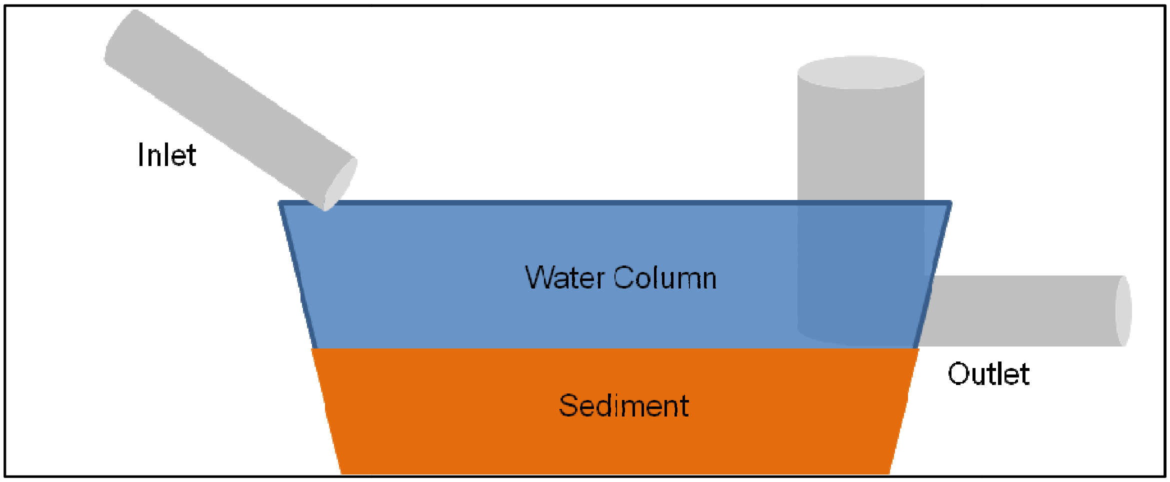

Figure 1.10 - Conceptualized graphic showing sample collection locations. Grab samples were taken at sediment basin inlets and outlets. To address spatial variability, composite samples were collected for basin water column and sediments.

To obtain sediment-associated *E. coli* densities, composite samples were collected from each pond substrate (Sediment) using a fabricated device constructed of a 25 cm section of perforated metal pressure pipe, two sections of 20 mm clear flexible plastic tubing and a 2.5 m^3/hour multipurpose drill pump. The pipe/hose section was deployed and drawn along the bottom from multiple representative basin transects (Figure 1.11). The slurry mix was pumped into a 20 liter carboy, vigorously agitated, and a single 500 ml sample was drawn and labeled sediment-water composite (SWC). The resulting aliquot was transferred to the laboratory for analysis.

Figure 1.11 - Deployment of fabricated sediment collection device at AHC site. Drill pump is shown drawing slurry mix into 20 liter carboy.

E. coli samples were processed within 6 hours of collection using the Colilert® enzyme substrate assay procedure, Method 9223 B, from Standard Methods for the Examination of Water and Waste Water (APHA, 2005). Concentrations for *E. coli* are reported as most probable number per 100 ml (MPN/100 ml). In addition to bacterial enumeration, all water samples were analyzed for TSS using Standard Method 2540 D (APHA, 2005).

Determination of sediment-associated *E. coli* density was made through laboratory procedure and corresponding unit balance computation. After removing a designated volume for bacterial enumeration, the remaining sediment composite sample was transferred to a graduated cylinder and allowed to settle for 24+ hours (Figure 1.12). Total volume of sample, volume of water, and volume of settled sediment and associated interstitial (pore) water were recorded. Sediment-associated *E. coli* concentrations were calculated using the following unit balance equation:

$$[EC]_{sed} = \frac{([EC]_{SWC} \times Vol_{SWC}) - ([EC]_{WC} \times Vol_{W})}{Vol_{sed}}$$

(Jolley, 2005)

where $[EC]_{sed}$, $[EC]_{swc}$, and $[EC]_{wc}$ are the *E. coli* concentrations for the sediment-associated bacteria, the sediment-water composite, and the water column, respectively; and Vol_{swc}, Vol_{w} and Vol_{sed} are the total volume of sediment-water composite, volume of water in graduated cylinder and volume of settled sediment and interstitial water, respectively. *E coli* densities are reported as MPN/100 ml.

Figure 1.12 - Graduated cylinders with collected bottom sediments. Total volume and volume of water and settled sediments are recorded for use in unit balance computation.

A primary focus of this research was to assess whether construction-derived sediment and basins designed to control the eroded material were contributing to bacterial contamination above recommended threshold concentrations. Thus, for relevant statistical analysis, *E. coli* densities are compared, where designated, with the single sample criteria for contact recreation of 235 MPN/100 ml, which was established and subsequently reaffirmed by the US Environmental Protection Agency (US EPA, 1986). Spatial independence was assumed for the sites because: 1) there was no surface water hydrologic connection between any basins, 2) contributing soil associations at each site varied by proportion of soil series, 3) each site underwent different grading and compaction procedures, and 4) previous land use varied by site. Statistical calculations were performed using SAS 9.2 (SAS, 2008). The statistical significance level was set at an alpha value of ≤ 0.05 unless otherwise stated.

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CHAPTER TWO

EVALUATION OF *ESCHERICHIA COLI* DENSITY IN CONSTRUCTION SITE RUNOFF AND SEDIMENT BASIN DISCHARGE IN THE PIEDMONT ECOREGION OF SOUTH CAROLINA

Written and formatted for submittal to:

JOURNAL OF THE AMERICAN WATER RESOURCES ASSOCIATION

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ABSTRACT

One of the primary practices utilized for treating construction derived runoff is a sediment retention basin. Sediment-laden flow is routed from areas undergoing active land disturbance to a newly excavated basin where larger particles settle out of suspension. Eight (8) construction projects in northwestern South Carolina and their associated sediment basins were monitored during the spring, summer and autumn of 2008 to quantify and evaluate related *Escherichia coli* (*E. coli*) densities. Dry and wet-weather samples were collected from basin inlets, outlets, water column and deposited sediments. Although variable, bacterial concentrations from construction site runoff (mean = 771 MPN/100 ml) were consistently and significantly higher than water quality criteria of 235 CFU/100 ml established by the US Environmental Protection Agency. Basin discharge showed significantly higher bacterial concentrations (mean = 1,368 MPN/ 100 ml; t-stat = 3.54; p = .0036; n = 14) than those coming off the construction sites. Within sediment basins, both mean water column (877 MPN/100 ml) and mean sediment (188,828 MPN/100 ml) *E. coli* densities were higher than recommended EPA criteria, with mean concentrations in the sediments significantly exceeding the corresponding overlying water column (t-stat = 5.51; p <0.0001; n = 73). The combined data suggest these sediment control practices are not acting to reduce bacterial concentrations, but rather they appear to be serving as reservoirs for viable *E. coli* and net sources of bacterial

loading to receiving waters. Basin data were also analyzed using Pearson correlation between *E. coli* densities, total suspended solids (TSS) and rainfall depth. Across sites and sample locations, *E. coli* displayed a weak but significant correlation with TSS (r = 0.23, p = 0.02) and with rainfall (r = 0.24, p $<$0.001). Quantification of construction site bacterial runoff, basin-related bacterial contamination, and examination of site discharges will assist stormwater regulators and engineers in evaluating the efficacy of state construction site permit standards, confirm whether site design practices required by regulatory agencies are protective of receiving water quality, and if construction stormwater design and management are suitable or sustainable.
(KEY TERMS: *Escherichia coli*; water quality criteria; construction site runoff; nonpoint source pollution; sediment detention basin; bacteria.)

INTRODUCTION

Construction-related land disturbance within the rapidly developing Piedmont region of the Southeastern United States affects thousands of hectares each year. Despite protective practices, excess sediment from these activities is deposited within creeks, rivers, lakes, and other nearby surface waters, adversely affecting the habitat of important aquatic species, reducing water clarity and transporting other potentially harmful pollutants such as adsorbed *Escherichia coli* (*E. coli*). Determining relationships between the presence, transport and fate of sediment-associated waterborne bacteria is of primary concern in South Carolina as the 2008 Section 303(d) list of impaired waterbodies indicates more contamination from fecal coliform than any other single pollutant (SC DHEC, 2008).

Bacteriological water quality criteria have existed for decades within the United States as well as other developed nations. Such standards are largely based on estimates of indicator species counts correlated with gastrointestinal illness rates (US EPA, 1986). Each year in the United States, occurrence of food- and water-borne illness related to certain specific pathogens has measurable economic impacts associated with medical costs and decreases in job productivity. In 2007 alone, an estimated $460M was lost resulting from a single strain of shiga toxin-producing *E. coli* (Frenzen, 2007). Establishing, maintaining or upgrading monitoring protocols is essential to protecting public health and economic well-being.

Bacteria are principle components of naturally occurring carbon and nutrient cycling in the environment. Genotypic and phenotypic diversity allows these organisms to survive under a broad range of physical, chemical and biological conditions (Winfield and Groisman, 2003; Maier *et al*., 2000). Ishii and Sadowsky (2008) suggest the capacity of certain enteric bacterial species like *E. coli* to survive long-term outside a host environment is likely due to their ability to acquire energy by various means. In essence, *E. coli* can become “naturalized” into the broader microbial community because it can exist under aerobic and anaerobic conditions, survive in a variety of temperatures, while needing only simple nutrients and trace elements to grow (Whitman *et al.*, 2006; Whitman *et al.*, 2004; Byappanahalli *et al.*, 2003; An *et al.*, 2002; Gagliardi *et al.*, 2002).

Research has shown that if established in the natural environment, *E. coli* can persist throughout the year, serving as a continuous bacterial source (Whitman *et al*., 2006; Byappanahalli *et al*., 2003; Gagliardi *et al*., 2002). Because a significant fraction of bacteria are associated with soil, runoff laden with newly eroded and suspended sediment can serve as a secondary source of higher *E. coli* concentrations to receiving-waterbodies (Wu *et al*., 2009; Jamieson *et al*., 2005). Characklis *et al*. (2005) found microbial adsorption varies by microorganism, with 20-30% of viable *E. coli* showing consistent affinity for settleable particle sizes.

Once mobilized, the fate of sediment-associated bacteria is determined in large measure by site-specific hydrologic conditions. Existing research has shown a significant relationship between bacterial concentration and both bottom and suspended sediments (Jamieson *et al*., 2005; Davies and Bavor, 2000; LaLiberte and Grimes, 1981). In stream systems it has been shown that elevated densities of *E. coli* during storm flow were not simply due to new bacteria entering via runoff, but also resulted from resuspension of sediment-associated bacteria within the channel (McDonald *et al*., 1982).

Recent regionally-focused research confirmed significant correlation between elevated sediment loads and correspondingly high concentrations of fecal coliform bacteria in S.C. Piedmont stream systems (Jolley, 2005). Jolley determined indicator bacteria and other waterborne pathogens adsorbed to sediment particles may survive following deposition, and further, that bacteria existing within this substrate environment can be resuspended and transported following perturbation. Certain pathogenic bacteria in bottom sediments have been found to survive significantly longer than populations found in overlying water columns (Burton *et al.*, 1987). Burton *et al*. also showed *E. coli* survival in sediments was related to smaller particle size and high clay content due to the presence of associated organic matter and nutrients as well as protection from predation within macropore spaces.

In lentic systems, bottom sediments have also been shown to act as potential bacterial reservoirs (Davies and Bavor, 2000; Howell *et al*., 1996;

LaLiberte and Grimes, 1981). Davis *et al*. (2005) concluded that pond sediments can sustain viable populations of *E. coli* for several months with no external input, and further, these bacteria may be resuspended back into the water column by turbulent flow associated with storms.

Beyond sediment transport and hydraulics modeling, construction site sediment basins are poorly understood and have not been the subject of noteworthy ecologically focused research. Despite design advances such as forebays and serpentine baffle structures, construction site sediment ponds in South Carolina are only required to capture 80% of total suspended solids (TSS) to meet the settleable solids criteria of the Stormwater Standards and Sediment Reduction Regulation (SCDHEC, 2002). Designed as either wet or dry systems, these basins are thus constructed to trap most entering sediment, but still release a proportional volume into nearby surface waters.

Given the association between eroded soils, bottom sediments and the ubiquitous nature of enteric bacteria in natural ecosystems, the objectives of this research were to: 1) evaluate *E. coli* densities in construction-derived stormwater runoff with respect to water quality criteria established by the US Environmental Protection Agency, and; 2) assess whether these basin systems created for controlling sediment and stormwater in the Piedmont region of South Carolina are acting as sources, sinks or reservoirs for potential pathogens.

FIELD SITES

Data were collected from eight (8) construction site sediment basins associated with permitted land disturbance activities in Anderson, South Carolina (Figure 2.1). Located in the Piedmont physiographic province, site soils are characterized primarily by the Cecil-Pacolet association, which are moderately to well-drained clay loams having dominantly clay subsoil (USDA-SCS, 1993). Mean annual precipitation is 127 cm and mean annual temperature is 16.3℃. Basin drainage areas varied from 0.95-19.2 hectares, with previous land uses including pasture, forested and mixed-use commercial. Individual basin storage for the 2-year, 24-hour design storm ranged from 730-8,733 m^3.

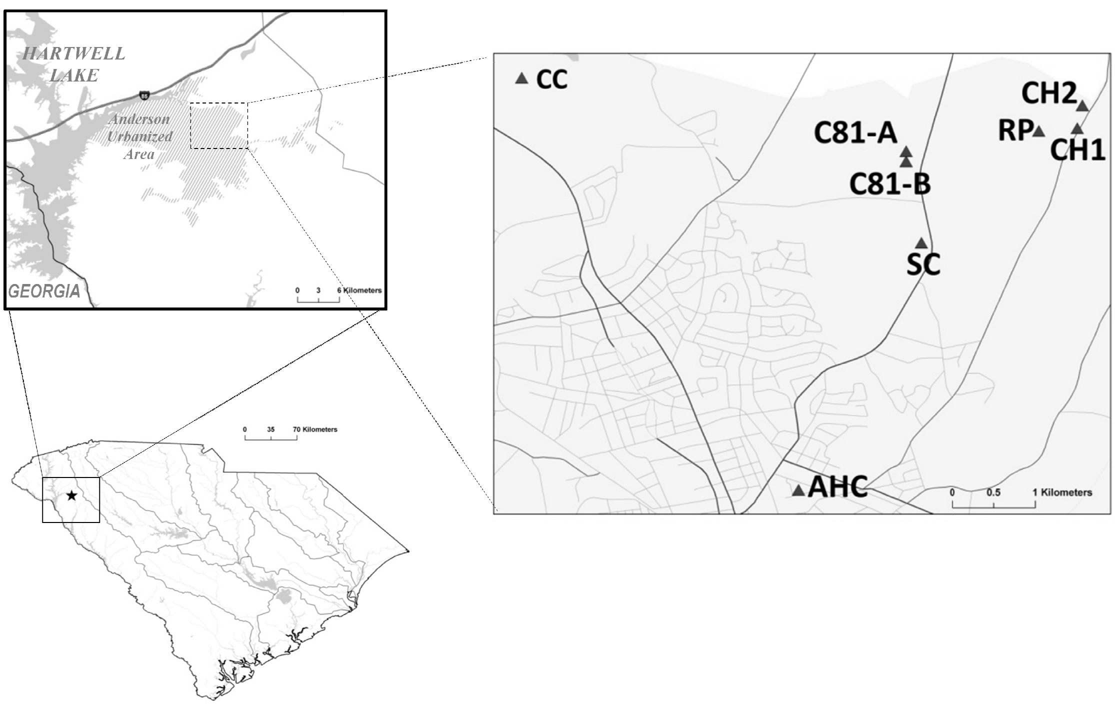

Figure 2.1 - Location of study area. Eight construction site sediment basins were dry- and wet-weather sampled from March-October of 2008. Site designations are shown on right.

METHODS

To evaluate changes in bacterial density over time within basins, the frequency for sampling was established to be every 7 calendar days in addition to each rain event which produced runoff. Since the time of concentration for these relatively small catchments was on the order of minutes, it was important to be on site to collect grab samples whenever a runoff event occurred. Physical and chemical parameters collected within the water column included pH, dissolved oxygen (DO), conductivity, temperature, rainfall, and days since last rainfall (DSLR).

For each sediment basin, there were 4 possible sample locations (Figure 2.2). During runoff events, 500 ml samples were collected at the basin inlet pipe, which led from the active construction site to the detention pond (Inlet). Whenever flowing, samples were also collected at the point of basin discharge (Outlet). Water column (WC) samples were obtained using a composite of 500 ml samples collected at representative locations (near outlets, inlets, and perimeter) using a long-handled polyethylene dipper and conveyed into an autoclavable 20 liter carboy. Contents were shaken vigorously for 60 seconds, and a single 500 ml sub-sample was drawn into a sterile bottle.

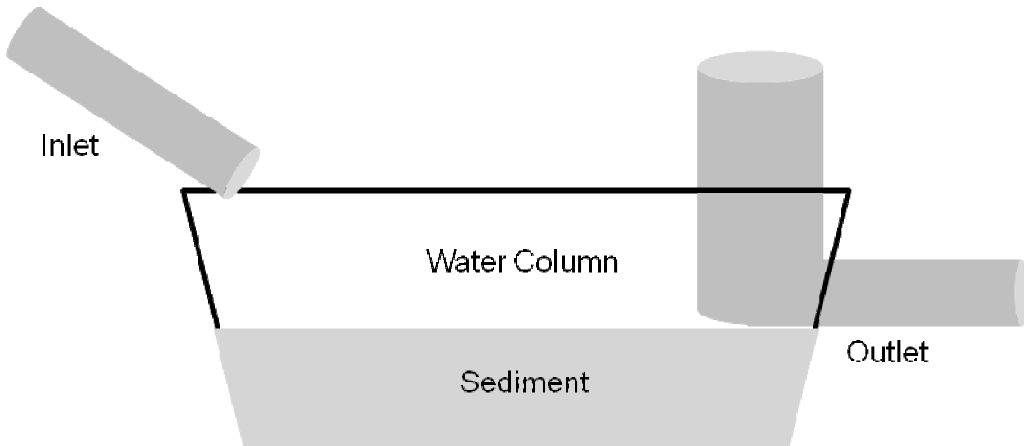

Figure 2.2 - Conceptualized graphic showing sample collection locations. Grab samples were taken at sediment basin inlets and outlets. To address spatial variability, composite samples were collected for basin water column and sediments.

To obtain sediment-associated *E. coli* densities, composite samples were collected from each pond substrate (Sediment) using a fabricated device constructed of a 25 cm section of perforated metal pipe, two sections of 20 mm clear flexible plastic tubing and a 2.5 m^3/hour multipurpose drill pump. The pipe/hose section was deployed and drawn along the bottom from multiple representative basin transects. The slurry mix was pumped into a 20 liter carboy, vigorously agitated, and a single 500 ml sample was drawn and labeled sediment-water composite (SWC). The resulting aliquot was transferred to the laboratory for analysis.

E. coli samples were processed within 6 hours of collection using the Colilert® enzyme substrate assay procedure, Method 9223 B, from Standard Methods for the Examination of Water and Waste Water (APHA, 2005). Concentrations of *E. coli* are reported as most probable number per 100 ml

(MPN/100 ml). In addition to bacterial enumeration, all water samples were analyzed for TSS using Standard Method 2540 D (APHA, 2005).

Determination of sediment-associated *E. coli* density was made through laboratory procedure and corresponding computational analysis. After removing a designated volume for bacterial enumeration, the remaining sediment composite sample was transferred to a graduated cylinder and allowed to settle for 24+ hours. Total volume of sample, volume of water, and volume of settled sediment and associated interstitial (pore) water were recorded. Sediment-associated *E. coli* concentrations were calculated using the following unit balance equation:

$$[EC]_{sed} = \frac{([EC]_{SWC} \times Vol_{SWC}) - ([EC]_{WC} \times Vol_{W})}{Vol_{sed}}$$

(Jolley, 2005)

where $[EC]_{sed}$, $[EC]_{swc}$, and $[EC]_{wc}$ are the *E. coli* concentrations for the sediment-associated bacteria, the sediment-water composite, and the water column, respectively; and Vol_{swc}, Vol_{w} and Vol_{sed} are the total volume of sediment-water composite, volume of water in graduated cylinder and volume of settled sediment and interstitial water, respectively. *E coli* densities are reported as MPN/100 ml.

A primary focus of this research was to assess whether construction-derived sediment and basins designed to control the eroded material were contributing to bacterial contamination above recommended threshold concentrations. Thus, for relevant statistical analysis, *E. coli* densities are compared, where designated, with the single sample criteria for contact recreation of 235 MPN/100 ml, which was established and subsequently reaffirmed by the US Environmental Protection Agency (US EPA, 1986). Statistical calculations were performed using SAS 9.2 (SAS, 2008). Spatial independence was assumed because: 1) there was no surface water hydrologic connection between any basins, 2) contributing soil associations at each site varied by proportion of soil series, 3) each site underwent different grading and compaction procedures, and 4) previous land use varied by site. The statistical significance level was set at an alpha value of ≤ 0.05 unless otherwise stated.

Of the 8 basins sampled, the CC site was significantly different than the others from both a design and hydrologic perspective. The basin was excavated to an elevation which supported significant groundwater influence. While all other basins were empty during one 40-day period without precipitation, the CC site continued to maintain a consistent depth and continuous discharge. Groundwater has been shown to contain negligible quantities of *E. coli* (Byappanahalli *et al*., 2003) and was clearly inserting a hydrological bias into results by diluting the water column and subsequent basin discharge. Mean outlet *E. coli* density for CC was 273 MPN/100 ml (n=28), and, for all other sites combined, the mean was 1368 MPN/100 ml (n=29). Nevertheless, when CC data were included in an independent samples t-test with remaining site outlet numbers, there was still evidence to conclude that *E. coli* densities in sediment basin discharge significantly exceed single sample contact recreation water quality criteria (mean = 829 MPN/100 ml; t-stat = 4.85; $p < .0001$; $n = 57$). For reasons stated above however, CC site data were not included in further statistical analysis.

RESULTS AND DISCUSSION

Analysis of grab and composite samples collected throughout the period of research indicate conditions associated with monitored sediment basins may pose a risk of microbial contamination to relevant receiving waterbodies. So that results across all dates and sites by sample location can be displayed in the same figure, *E. coli* density data were log-transformed and shown in Figure 2.3.

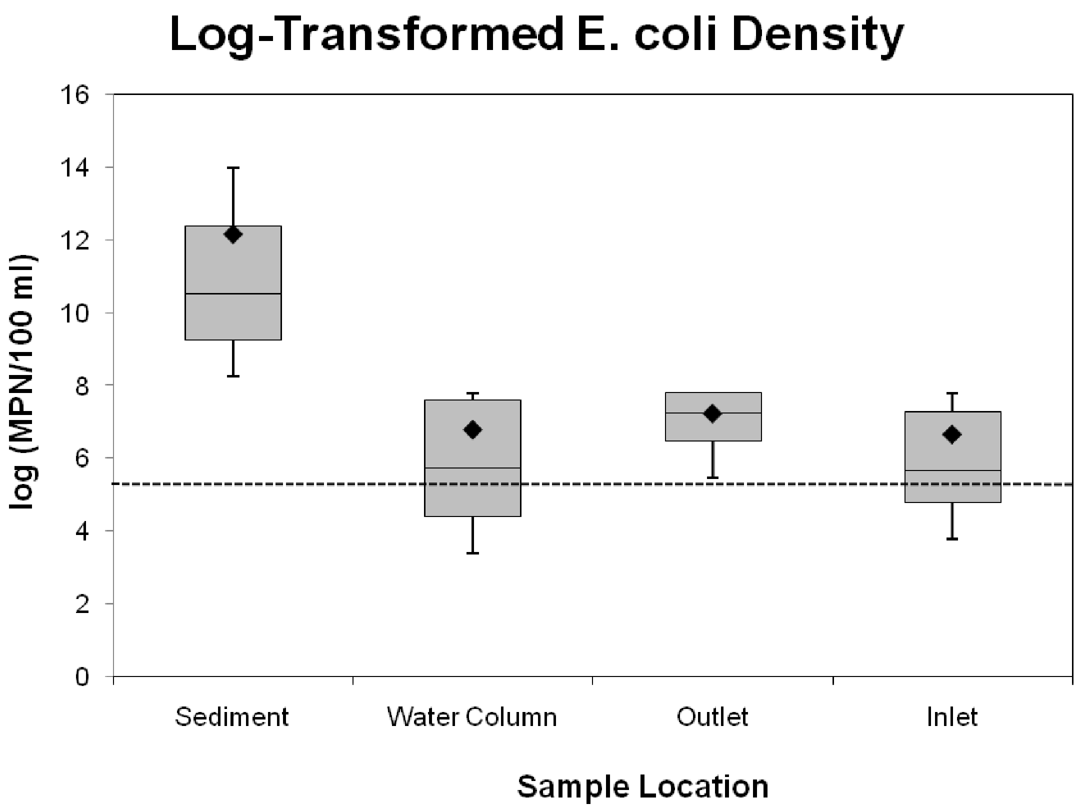

Figure 2.3 - Box and whisker plot of log-transformed mean E. coli densities (MPN/100 ml) across dates and sites for each sample location. A dashed reference line is drawn at 5.86 (log-transformed value of 235), which is the EPA-recommended water quality criteria for contact recreation. Eighty-one percent (81%) of all observations exceeded the criterion threshold.

Site-specific *E. coli* density means across dates for water samples (inlet, outlet, water column) exhibited substantial variability (Figure 2.4). Inlet means ranged from a low of 114 MPN/100 ml at C81-B inlet to a high of 1728 MPN/100 ml at the RP outlet. Sediment-associated *E. coli* means were several orders of magnitude higher than those of their corresponding water columns, and ranged from a low of 37,103 MPN/100 ml at AHC to a high of 488,923 MPN/100 ml at CH1. As the water quality criteria reference line indicates in Figure 2.4, only one sampling location (C81-B inlet) of all sites evaluated fell below the 235 MPN/100 ml recommended threshold. While measures of central tendency are useful, means testing alone did not provide the comparative results necessary to answer primary questions.

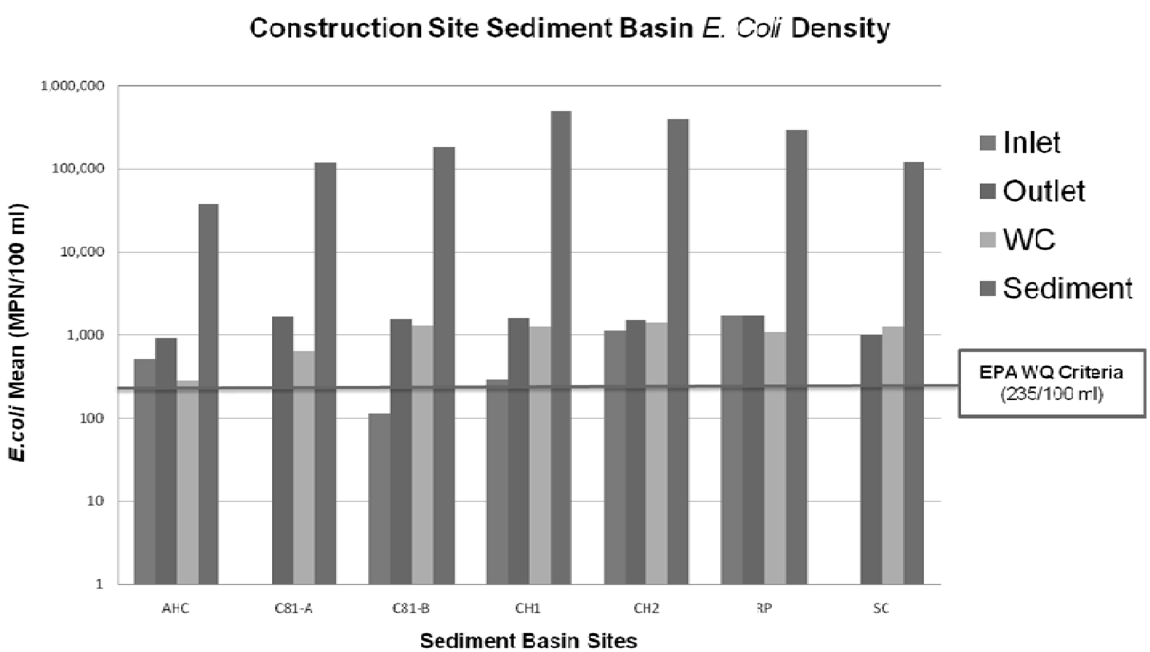

Figure 2.4 - *E. coli* density means by site and sample location. Missing inlet data for C81-A and SC were a function of pipe occlusion resulting in inlet-water column mixing.

Inlet Results

To determine bacterial contribution from the construction sites, inlet *E. coli* densities were isolated and analyzed. The intent of this aspect of the research was to quantify and test whether there was a significant difference between recommended EPA criteria and *E. coli* densities measured from construction site runoff. T-test results indicate the true mean for inlet samples across sites and dates was significantly greater than 235 (mean = 771 MPN/100 ml; t-stat = 2.16; $p = 0.025$; $n = 14$). Since the Shapiro-Wilk test suggested the data were not normal, the hypothesis was also subjected to the Wilkoxon Signed-Rank test (S-stat = 39.5; $p = 0.0106$) which confirmed the result.

The relatively low number of inlet samples ($n = 14$) is partially a function of inadequate basin maintenance. At the SC and C81-A sites, inlet inverts were beneath deposited sediment, occluding the pipes and prohibiting the possibility of collecting samples not compromised by backflow from the pond. Further, although the number of samples for this analysis is relatively small, the data represent multiple storm dates across multiple sites.

Outlet Results

To quantify *E. coli* densities in sediment basin discharge, outlet data were similarly tested against EPA criteria. T-test results showed mean *E. coli* densities for outlet samples collected across dates and sites was significantly greater than 235 (mean = 1368 MPN/100 ml; t-stat = 6.70; $p < 0.0001$; $n = 29$).

Again, the Shapiro-Wilk test indicated a statistically significant departure from normality for the outlet data so the hypothesis was also subjected to the Wilkoxon Signed-Rank test (S-stat = 199.5; $p < 0.0001$).

These data indicate *E. coli* was present at evaluated construction sites and entered sediment basins at concentrations exceeding EPA recommended thresholds. It is also evident *E. coli* concentrations in sediment basin discharge also significantly exceed recommended values. To assess the potential difference between *E. coli* densities found in construction site runoff and those found in sediment basin discharge, a paired samples t-test was employed. Results confirmed that *E. coli* concentrations in basin discharge were significantly higher than corresponding concentrations in site runoff (t-stat = 3.54; $p = 0.0036$; $n = 14$). Thus construction site sediment basin systems appear to be acting as reservoirs for *E. coli* in addition to serving as net sources of bacterial loading to receiving waters.

E. coli Association with Sediments

Analysis was conducted on basin water column and sediment-associated *E. coli* densities, confirming both contained E. coli densities significantly above the recommended EPA water quality threshold. Independent samples t-tests on water column data yielded a mean across sites and dates of 877 MPN/100 ml (t-stat = 5.70; $p < 0.0001$; $n = 73$) while sediment-associated data provided a mean of 188,828 MPN/100 ml (t-stat = 5.51 and $p = < 0.0001$; $n = 73$). A paired samples t-test confirmed *E. coli* concentrations were significantly higher in the sediments than in the overlying water column (t-stat = 5.53; $p < 0.0001$; n = 73). Site averages for sediment-associated *E. coli* varied by an order of magnitude yet all site means were in excess of 37,103 MPN/100 ml (Table 2.1). Although statistical tests prove neither growth nor decay, they confirm an abundant reservoir of *E. coli* available for resuspension given the necessary physical conditions.

TSS means varied by sample location (Table 2.1). As expected, TSS from the construction sites as measured from the basin inlets provided the highest mean (166.1 mg/l; n = 14), followed by outlets (120.5 mg/l; n = 29) and water column (94.9 mg/l; n = 73). However, paired sample t-test results indicated there was not enough evidence that TSS concentrations in basin discharge were significantly lower than TSS concentrations in site runoff (t-stat = 0.93; p = 0.370) for the basins and storms sampled.

TABLE 2.1 - Means analysis for all sediment basin sites and sample locations

			Measured Variables							
Site	Sample Location	*n*	*E. coli* Density (MPN/100 ml)	TSS (mg/l)	Rainfall (cm)	DSLR[a] (days)	Temp (℃)	pH	DO (mg/l)	Cond (µS/cm)
AHC	WC	14	282 +/- 587	106.3	1.40	2.11	26.0	6.3	6.1	177.0
	Inlet	5	515 +/- 727	332.3	2.01	0.10				
	Outlet	7	913 +/- 1,146	151.2	2.67	0.29				
	Sediment	14	37,103 +/- 60,539		1.40	2.11				
C81-A	WC	21	627 +/- 783	92.7	0.96	2.48	28.7	5.8	5.0	59.4
	Inlet				0.00					
	Outlet	5	1,642 +/- 846	147.7	3.07	0.30				
	Sediment	21	117,488 +/- 188,477		0.97	2.48				
C81-B	WC	5	1,297 +/- 696	75.2	1.85	0.60	26.4	5.3	5.3	47.8
	Inlet	2	114 +/- 91	17.7	3.19	0.00				
	Outlet	4	1,551 +/- 662	33.7	2.32	0.00				
	Sediment	5	180,124 +/- 194,105		1.85	0.60				
CH1	WC	6	1,274 +/- 1,257	106.4	2.49	0.50	27.7	5.7	5.6	94.9
	Inlet	2	289 +/- 5	81.1	2.76	0.00				
	Outlet	2	1,594 +/- 1,166	153.3	2.76	0.00				
	Sediment	6	488,923 +/- 423,009		2.49	0.50				
CH2	WC	6	1,405 +/- 1,148	82.7	2.49	0.50	27.8	5.3	5.2	59.2
	Inlet	2	1,123 +/- 1,221	193.0	2.76	0.00				
	Outlet	2	1,534 +/- 1,252	82.1	2.76	0.00				
	Sediment	6	390,654 +/- 398,179		2.49	0.50				
RP	WC	12	1,084 +/- 1,106	69.0	1.63	1.63	27.8	5.7	4.4	59.6
	Inlet	3	1,721 +/- 1,209	26.8	2.56	0.00				
	Outlet	5	1,728 +/- 717	125.6	2.17	0.10				
	Sediment	12	295,038 +/- 427,261		1.63	1.63				
SC	WC	9	1,254 +/- 1,033	128.6	2.08	1.72	26.4	5.5	4.8	61.7
	Inlet				0.00					
	Outlet	4	989 +/- 972	115.6	2.49	0.13				
	Sediment	9	119,914 +/- 128,968		2.08	1.72				

E. coli density values shown as MPN/100 ml +/- standard deviation

[a] Days since last rainfall; *n* , number of samples

Pearson correlation between TSS and *E. coli* density across all sites, dates and sample locations yielded a significant positive, but weak result (r = 0.21; p = 0.022; n = 116). Correlations by sample location across dates and sites provide more revealing results. For both water column (r = 0.42; p = 0.0002; n =

73) and outlet (r = 0.27; p = 0.15; n = 29), correlations between bacteria and suspended sediment were moderate to weak, but in the positive direction. For runoff leaving the construction sites as measured at the inlet, the Pearson correlation between TSS and *E. coli* density was moderate but negative (r = -0.42; p = 0.13). While not statistically significant, this difference may be a function of eroded size distribution for Cecil soils leaving the site, gravitational sorting occurring within the water column and preferential association of bacteria with smaller and lighter, clay-sized particles.

CONCLUSIONS

Construction site sediment basins have been poorly understood with respect to the potential for bacterial contamination. Yet these hydrologic systems are routinely built throughout the United States to address regulatory stormwater permitting requirements. Current modeling efforts to support development of total maximum daily loads (TMDLs) do not account for bacteria allocations associated with construction activities. Yet these land-disturbing activities have physical impacts that occur at multiple spatial and temporal scales.

With such a large reservoir of viable *E. coli* associated with bottom sediments (mean = 188,828 MPN/100 ml), remobilization during relatively turbulent rain events seems likely. Though mean TSS was lower at outlets compared with inlets, preferential association of *E. coli* with smaller clay particles discharged through the site outlet could explain the correspondingly elevated bacterial concentrations. Ehrhart *et al.* (2002) demonstrated that preferential settling within sediment basins of larger eroded particles produced effluent containing a higher proportion of finer suspended sediments downstream, as measured by particle size distribution. Controlled research over 8 years conducted in experimental sediment basins found that on average, 24% of sediment lost through discharge represented resuspension of previously deposited bottom sediments (Jarrett, 2001; Fennessey and Jarrett, 1996).

Bacterial source tracking was beyond the scope of this project. Nonetheless on a strictly qualitative basis, evidence of possible sources was routinely observed and photographically documented. Various feces deposited directly within basin catchments were visually confirmed as deer, raccoon, bird, cat and dog. Animal tracks both entering and exiting areas subject to storm-related inundation were also detected on a regular basis. In addition, 3 of the sites were hydro-seeded either during, or previous to, the onset of sampling. Aside from grass seed and surfactant, these mixtures often contain "proprietary" blends of fertilizer, including manure.

Direct fecal input however, does not sufficiently account for the observed difference in bacterial density between bottom sediments and those of the overlying water column or corresponding basin discharge. While additional research should undertake a mass balance accounting of water and sediments, it appears likely that *E. coli* is persisting for months within basin substrates. Such findings would likely confound ongoing construction-related regulatory policy.

Regardless of original source, Whitman et al. (2006) found *E. coli* was abundant in undisturbed soils, streams and interstitial water. Results of research generated through this project, while focused on construction-derived soils and associated man-made hydrologic systems, would appear to confirm Whitman's findings for evaluated sediment basins. *E. coli* concentration means from all sample locations (inlet, outlet, water column and sediments) significantly exceeded values recommended by EPA for safe contact recreation. While no author is suggesting construction site sediment basins are utilized for recreation, these impoundments discharge to downstream surface waters which often serve those roles for public use.

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CHAPTER THREE

ABUNDANCE AND DISTRIBUTION *OF ESCHERICHIA COLI* ASSOCIATED WITH CONSTRUCTION SITE SEDIMENT BASINS

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ABSTRACT

Escherichia coli are found in the intestinal tract of warm blooded animals, and as such are widely utilized by the regulatory community to indicate the potential presence of fecal pathogens. Recent research however, suggests these microorganisms survive and persist under wide-ranging conditions outside a host or primary environment. To build on the findings of such research, 7 construction sites in northwestern South Carolina were monitored during the spring, summer and autumn of 2008 to quantify *E. coli* densities and evaluate their distribution and movement through the sediment basin system. Dry and wet-weather samples collected from basin inlets, outlets, water column and deposited sediments indicated *E. coli* counts significantly above levels recommended by EPA for contact recreation. Mean *E. coli* densities were highest in deposited sediments (188,828 MPN/100 ml), followed by basin outlets (1368 MPN/ 100ml), water columns (877 MPN/100 ml) and basin inlets (771 MPN/100 ml). Among sample locations and across dates, significant correlations were found between *E. coli* concentrations and TSS, pH, temperature and rainfall depth. The authors conclude that: (i) *E. coli* are present at significant concentrations in construction-derived sediments; (ii) rainfall depth appears to significantly influence E. coli densities within these man-made hydrologic systems; (iii) construction site sediment basins in the Piedmont of South Carolina are serving as reservoirs of viable *E. coli;* and (iv) resuspension of subsequent

transport of bottom sediments during storm events help account for the persistently elevated *E. coli* levels in sediment basin discharge.

INTRODUCTION

Bacteriological water quality criteria have existed for decades within the United States as well as other developed nations. Such standards are largely based on estimates of indicator species counts correlated with gastrointestinal illness rates (24). Due in part to such high associations in freshwater and relative ease of enumeration, *E. coli* has proven to be a reliable predictor of waterborne illness. In response, EPA promulgated water quality criteria for primary contact recreation having a geometric mean of 126 CFU/100 ml for samples collected over a 30-day period or 235 CFU/100 ml for any single sample. Each year in the United States, occurrence of food- and water-borne illness related to monitored pathogens also has measurable economic impacts. In 2007 alone, an estimated $460M was lost in medical costs and job productivity resulting from a single strain of shiga toxin-producing *E. coli* (10).

Bacteria are principle components of naturally occurring carbon and nutrient cycling in the environment. Genotypic and phenotypic diversity allows these microorganisms to survive under a broad range of physical, chemical and biological conditions (18, 29). Ishii and Sadowsky (13) suggest the ability of certain enteric bacterial species like *E. coli* to survive long-term outside a host environment is likely due to their ability to acquire energy by various means. In essence, *E. coli* can become “naturalized” into the broader microbial community because it can exist under aerobic and anaerobic conditions, survive in a variety of temperatures, while needing only simple nutrients and trace elements to survive (2, 4, 7, 11, 13, 22, 29, 30).

Research has shown that if established in the natural environment, *E. coli* can survive throughout the year, serving as a continuous bacterial source (4, 11, 28, 29). Because a significant fraction of bacteria are associated with soil, runoff laden with newly eroded and suspended sediment can serve as a secondary source of higher *E. coli* concentrations to receiving-waterbodies (2, 14, 32). Characklis *et al*. (5) found microbial adsorption varies by microorganism, with 20-30% of viable *E. coli* showing consistent affinity for settleable particle sizes.

Once mobilized, the fate of sediment-associated bacteria is determined in large measure by site-specific hydrologic conditions. Recent research found significant correlation between elevated sediment loads and correspondingly high concentrations of fecal coliform bacteria in Piedmont stream systems (16). It was concluded that fecal coliform adsorbed to sediment particles survive following deposition, and that bacteria existing within this substrate environment can be resuspended and transported following perturbation. Certain pathogenic bacteria in bottom sediments have been found to survive significantly longer than comparable populations in overlying water columns (3).

In lentic systems, bottom sediments have also been shown to act as potential bacterial reservoirs (6, 12, 17, 19, 21). Davis *et al*. (7) recently concluded that pond sediments can sustain viable populations of *E. coli* for several months with no external input and further, these bacteria may be resuspended back into the water column by turbulent flow associated with storms.

Sediment retention basins are a broadly utilized best management practice to control construction-derived runoff. Sediment-laden flow is routed from areas undergoing active land disturbance to a newly excavated impoundment, where larger particles are provided an opportunity to settle under the influence of gravity. Compared with discharge from a standard outlet structure, cumulative sediment loss can be lowered by installation of alternative dewatering systems that increase flow path length and preferentially remove discharge from the top of the water column (9, 20, 24). Ongoing applied research over an 8 year period however, demonstrated that while efficient at removing a large proportion of the incoming sediment volume, on average, 24% of cumulative sediment loss from basins resulted directly from resuspension of previously deposited material (15).

Beyond sediment transport and hydraulics modeling, construction site sediment basins are poorly understood and have not been the subject of published ecologically focused research. Given the association between eroded soils, bottom sediments and the ubiquitous nature of enteric bacteria in natural ecosystems, the objectives of this research were to: (i) evaluate *E. coli* densities in construction-derived runoff in the Piedmont of South Carolina; (ii) assess whether these basin systems created for controlling sediment and stormwater in the region are acting as sources, sinks or reservoirs for potential pathogens; and

(iii) examine relationships between bacterial concentrations and observed environmental variables.

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MATERIALS AND METHODS

Data were collected from 7 construction site sediment basins associated with permitted land disturbance activities in Anderson, South Carolina (Figure 3.1). Located in the Piedmont physiographic province, mean annual precipitation is 127 cm, and mean annual temperature is 16.3℃. Site soils are characterized primarily by the Cecil series, which is a moderately to well-drained clay loam having dominantly clay subsoil and a clay content ranging between 5-35% (27). In all cases however, native site soils were subjected to substantive physical alteration, including compaction and grading.

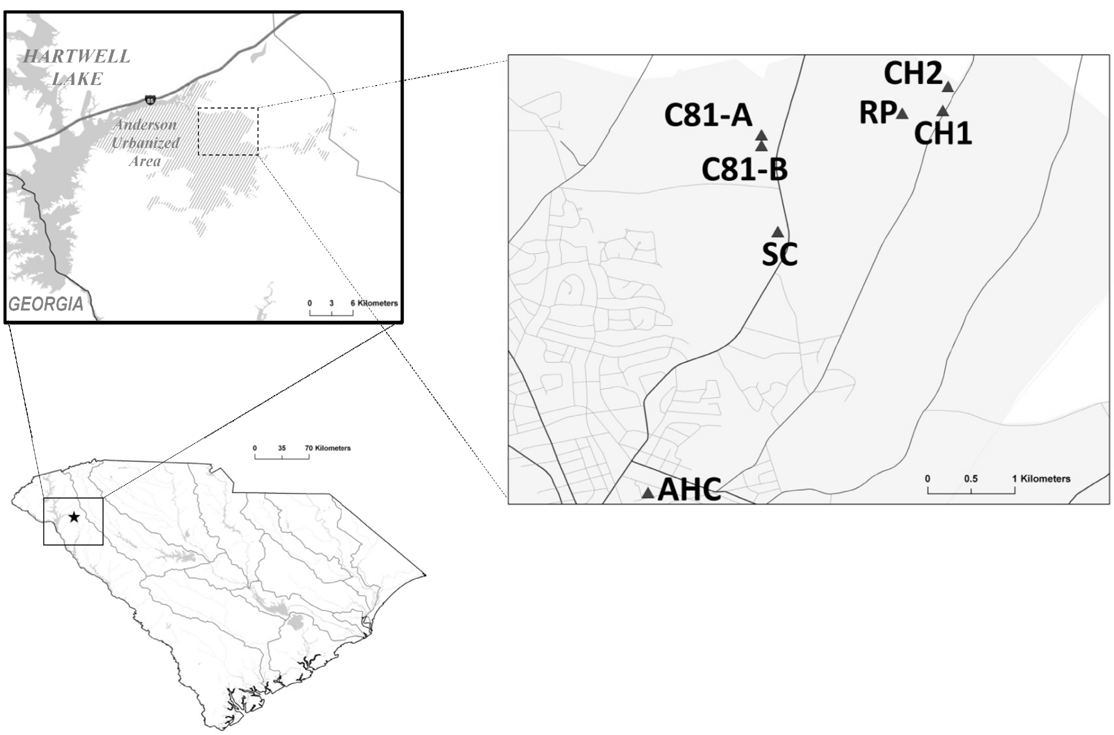

Figure 3.1 - Location of study area. Seven construction site sediment basins were dry- and wet-weather sampled from March-October of 2008. Site designations are shown on right.

Basin drainage areas varied from 0.95 - 19.2 ha, with previous land uses including pasture, forested and mixed-use commercial. Individual basin storage for the 10-year, 24-hour design storm ranged from 730 - 8,733 m^3. Basins ranged in age from brand-new to almost 14 months at the onset of sampling. From the newly excavated basins, samples were collected for each rain event that occurred during the course of this research. Spatial independence was assumed because: 1) there was no surface water hydrologic connection between basins, 2) contributing soil associations at each site varied by proportion of soil series, 3) each site underwent different grading and compaction procedures, and 4) previous land use varied by site.

To evaluate changes in bacterial density over time within basins, the frequency for sampling was established to be every 7 calendar days in those basins containing surface water in addition to any rain event which produced runoff. Since the times of concentration for these relatively small catchments typically occurred within minutes, it was important to be on site to collect samples whenever a runoff event took place. Complicating sample collection protocol, design specifications typically require sediment basins to be completely dewatered within a number of days (8, 9).

To assess potential predictive values and evaluate the relative importance of various physical and chemical parameters using bivariate correlation and regression analysis, 8 variables were observed. In addition to *E. coli* density, measurements were made for total suspended solids (TSS), pH, dissolved oxygen (DO), conductivity, temperature, rainfall depth, and days since last rainfall (DSLR).

For each sediment basin, there were 4 possible sample locations (Figure 3.2). During runoff events, 500 ml samples were collected at basin inlet pipes, which lead from the active construction site to the detention basin (Inlet). Whenever flowing, samples were also collected at the point of basin discharge (Outlet). Water column (WC) samples were obtained using a composite of 500 ml sub-samples collected at representative locations (near outlets, inlets, and basin perimeter) using a long-handled polyethylene dipper and conveyed into an autoclavable 20 liter carboy. Contents were shaken vigorously for 60 seconds, and a single 500 ml sub-sample was drawn into a sterile bottle.

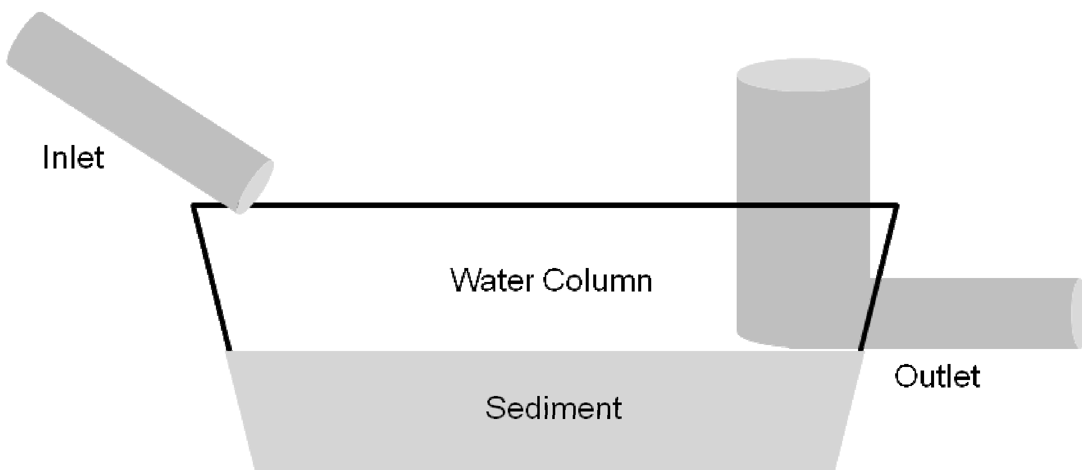

Figure 3.2 - Conceptualized graphic showing sample collection locations. Grab samples were taken at sediment basin inlets and outlets. To account for spatial variability, composite samples were collected within each basin water column and bottom sediments.

To obtain sediment-associated *E. coli* densities, composite samples were collected from each pond substrate (Sediment) using a fabricated device constructed of a 25 cm section of perforated metal pipe, two sections of 20 mm clear flexible plastic tubing and a 2.5 m^3/hour multipurpose drill pump. The pipe/hose section was deployed and drawn along the pond substrate from multiple representative transects. The slurry mix was pumped into a 20 liter carboy, vigorously agitated, and a single 500 ml sample was drawn and labeled sediment-water composite (SWC). The resulting aliquot was transferred to the lab for analysis.

E. coli samples were processed within 6 hours of collection using the Colilert® enzyme substrate assay procedure, Method 9223 B, from Standard Methods for the Examination of Water and Waste Water (1). Concentrations for *E. coli* are reported as most probable number per 100 ml (MPN/100 ml). In addition to bacterial enumeration, all water samples were analyzed for TSS using Standard Method 2540 D (1).

Determination of sediment-associated *E. coli* density was made through laboratory procedure and corresponding computational analysis. After removing a designated volume for bacterial enumeration, the remaining sediment composite sample was transferred to a graduated cylinder and allowed to settle for 24+ hours. Total volume of sample, volume of water, and volume of settled sediment and associated interstitial (pore) water were recorded. Sediment-associated *E. coli* concentrations were calculated using the following unit balance equation:

$$[EC]_{sed} = \frac{([EC]_{SWC} \times Vol_{SWC}) - ([EC]_{WC} \times Vol_{W})}{Vol_{sed}}$$

(16)

where $[EC]_{sed}$, $[EC]_{swc}$, and $[EC]_{wc}$ are the *E. coli* concentrations for the sediment-associated bacteria, the sediment-water composite, and the water column, respectively; and Vol_{swc}, Vol_w and Vol_{sed} are the total volume of sediment-water composite, volume of water in graduated cylinder and volume of settled sediment and interstitial pore water, respectively. Statistical calculations were performed using SAS 9.2 (23). The statistical significance level was set at an alpha value of ≤ 0.05 unless otherwise stated.

RESULTS

***E. coli* analysis from basin inlets**. Bacterial contribution from the construction sites was determined using *E. coli* densities measured at basin inlets. Raw data for all sites and sample locations, including site inlets is shown in Table 3.1. T-tests indicate the mean for inlet samples across sites and dates (771 MPN/100 ml) was significantly higher than the EPA recommended single sample water quality criterion of 235 MPN/100 ml (P = 0.025; $t_{0.05,13}$ = 2.16). Pearson analysis revealed only two predictor variables that were moderately correlated to *E. coli* density: TSS (r = -0.42, n = 14) and rainfall depth (r = 0.51, n = 14), though both failed to achieve significance, with P values of 0.13 and 0.06 respectively.

For construction site runoff data, multiple regression analysis also failed to find a linear model that significantly predicts *E. coli* density from any of the measured independent variables. The relatively weak coefficient of determination (r^2 = 0.28) corresponded to the indeterminate results of the significance test for the regression line equation ($F_{3,\,14}$ = 1.28, P = 0.34). Thus, while *E. coli* are present in evaluated construction site runoff at densities well above those recommended by the EPA for contact recreation, there fails to be a statistically reliable prediction model for suspended solids, rainfall depth and DSLR.

TABLE 3.1 - Means analysis for all sediment basin sites and sample locations

Site	Sample Location	*n*	*E. coli* Density (MPN/100 ml)	TSS (mg/l)	Rainfall (cm)	DSLR[a] (days)	Temp (℃)	pH	DO (mg/l)	Cond (µS/cm)
			Measured Variables							
AHC	WC	14	282 +/- 587	106.3	1.40	2.11	26.0	6.3	6.1	177.0
	Inlet	5	515 +/- 727	332.3	2.01	0.10				
	Outlet	7	913 +/- 1,146	151.2	2.67	0.29				
	Sediment	14	37,103 +/- 60,539		1.40	2.11				
C81-A	WC	21	627 +/- 783	92.7	0.96	2.48	28.7	5.8	5.0	59.4
	Inlet				0.00					
	Outlet	5	1,642 +/- 846	147.7	3.07	0.30				
	Sediment	21	117,488 +/- 188,477		0.97	2.48				
C81-B	WC	5	1,297 +/- 696	75.2	1.85	0.60	26.4	5.3	5.3	47.8
	Inlet	2	114 +/- 91	17.7	3.19	0.00				
	Outlet	4	1,551 +/- 662	33.7	2.32	0.00				
	Sediment	5	180,124 +/- 194,105		1.85	0.60				
CH1	WC	6	1,274 +/- 1,257	106.4	2.49	0.50	27.7	5.7	5.6	94.9
	Inlet	2	289 +/- 5	81.1	2.76	0.00				
	Outlet	2	1,594 +/- 1,166	153.3	2.76	0.00				
	Sediment	6	488,923 +/- 423,009		2.49	0.50				
CH2	WC	6	1,405 +/- 1,148	82.7	2.49	0.50	27.8	5.3	5.2	59.2
	Inlet	2	1,123 +/- 1,221	193.0	2.76	0.00				
	Outlet	2	1,534 +/- 1,252	82.1	2.76	0.00				
	Sediment	6	390,654 +/- 398,179		2.49	0.50				
RP	WC	12	1,084 +/- 1,106	69.0	1.63	1.63	27.8	5.7	4.4	59.6
	Inlet	3	1,721 +/- 1,209	26.8	2.56	0.00				
	Outlet	5	1,728 +/- 717	125.6	2.17	0.10				
	Sediment	12	295,038 +/- 427,261		1.63	1.63				
SC	WC	9	1,254 +/- 1,033	128.6	2.08	1.72	26.4	5.5	4.8	61.7
	Inlet				0.00					
	Outlet	4	989 +/- 972	115.6	2.49	0.13				
	Sediment	9	119,914 +/- 128,968		2.08	1.72				

E. coli density values shown as MPN/100 ml +/- standard deviation

[a] Days since last rainfall; *n*, number of samples

***E. coli* analysis from basin outlets.** Mean *E. coli* densities measured at basin outlets across all dates by site ranged from a low of 913 MPN/100 ml at AHC to a high of 1,728 MPN/100 ml at RP. T-test results indicate the mean for outlet samples across sites and dates (1368 MPN/100 ml) was also significantly higher than the EPA recommended criteria for contact recreation (P < 0.0001; $t_{0.05,\ 29}$ = 6.70).

E. coli densities were regressed against the observed independent variables in outlet data and showed only moderate correlation (r^2 = 0.34) with MPN ($F_{3,\ 25}$ = 4.25, P = 0.015). Beta weights were reviewed to assess relative importance of each variable in predicting *E. coli* densities. Both rainfall depth (β = 0.52, P = 0.0087) and DSLR (β = -0.49, P = 0.0208) provided standardized coefficients that indicated relatively high importance in terms of prediction within the outlet regression model.

To assess the difference between *E. coli* densities found in construction site runoff and those found in sediment basin effluent, data were subjected to a paired samples t-test. Results confirmed that *E. coli* concentrations in basin discharge were significantly higher than corresponding concentrations in site runoff (P = 0.0036, $t_{0.05,14}$ = 3.54). Thus construction site sediment basin systems appear to be acting as reservoirs for *E. coli* in addition to serving as net sources of bacterial loading to receiving waters.

***E. coli* analysis from basin bottom sediments.** Among the 3 sediment-related variables, only *E. coli* density and rainfall depth were significantly correlated (P < 0.0001, r = 0.50, n = 73) across all dates and sites. Sediment associated *E. coli* densities (n = 73) exhibited considerable range (1,182,186 MPN/100 ml), with a mean (188,828 MPN/100 ml), standard deviation (291,780 MPN/100 ml) and variance (8,513,590,746 MPN/100 ml) disproportionally high relative to those same statistics generated for the water-based sample locations at the inlet, outlet and water column.

Regression of MPN values against rainfall depth and DSLR yielded a weak but significant relationship (r^2 = 0.25, $F_{2,\,73}$ = 11.38, P < 0.0001). Standardized regression coefficients confirmed that rainfall, with a beta weight of 0.51 (P < 0.0001) was the only useful predictor variable for this model.

If only storm dates are used in the analysis, defined as when rainfall depth exceeded 0.5 cm, results of the linear regression model become more precise. Pearson correlation between *E. coli* density and rainfall depth is strengthened (P < 0.0001, r = 0.63, n = 45) and regression analysis yields a more significant relationship among the variables (r^2 = 0.44, $F_{2,\,45}$ = 16.18, P < 0.0001).

***E. coli* analysis from basin water columns.** Water column data involved the most complex analysis, due in large part to the increased number of variables when compared with other sample locations. Means, standard deviations, and Pearson correlations for water column data appear in Table 3.2. Bivariate correlations (n = 73) revealed five predictor variables that were significantly related to *E. coli* density: TSS (r = 0.42, P = 0.0002), temperature (r = -0.46, P < 0.0001), pH (r = -0.45, P < 0.0001), DSLR (r = -0.29, P = 0.012) and conductivity (r = -0.28, P = 0.019).

TABLE 3.2 - Means, standard deviations, and intercorrelations for sediment basin water columns across all dates and sites

Variable	M	SD	Intercorrelations 1	2	3	4	5	6	7
1. E. coli density (MPN/100 ml)	876.65	930.71							
2. TSS (mg/l)	94.94	72.12	42**						
3. Rainfall (cm)	0.28	0.75	15	07					
4. Temperature (℃)	27.46	3.87	-46**	-46**	-16				
5. pH	5.79	0.54	-45**	-24*	-38*	28*			
6. DSLR (days)	1.70	2.68	-29*	-36*	-45**	45**	41**		
7. Conductivity (µS/cm)	84.55	69.32	-28*	-20	-33*	01	58**	40**	
8. Dissolved oxygen (mg/l)	5.16	1.42	-8	04	07	11	21	06	31*

Note. n = 73. DSLR = Days since last rainfall. Decimals omitted from correlations.
*$p < .05$, **$p < .001$

Water column *E. coli* densities were then regressed against all measured variables. The resulting model was only moderately predictive ($r^2 = 0.43$, $F_{7, 67} = 6.21$, $P < 0.0001$). Standardized multiple regression coefficient analysis revealed that 3 of 7 variables were significant in their ability to predict *E. coli* counts. TSS had somewhat more influence (β = 0.34, $P = 0.0003$), but temperature (β = -0.29, $P = 0.0245$) and pH (β = -0.27, $P = 0.0432$) both demonstrated importance to the fit. In both instances, the coefficients were in the predicted direction.

Water column *E. coli* densities were also compared between storm (rainfall depth > 0.5 cm) and dry weather conditions (rainfall < 0.5 cm; DSLR > 1). Mean *E. coli* density for storm events was 1200 MPN/100 ml (n = 45) and for dry weather conditions the mean was only 356 MPN/100 ml (n = 28). An unpaired t-test confirmed that storm-related water column *E. coli* densities were significantly higher than those found during normal conditions ($t_{0.05, 71} = 4.46$, $P < 0.0001$). TSS data from the water column were also subjected to an unpaired t-test which further substantiated the difference between storm and dry-weather conditions ($t_{0.05,\,71}$ = 5.47, P < 0.0001).

Water column and sediment *E. coli* correlations. Independent samples t-tests on water column data yielded a mean across sites and dates of 877 MPN/100 ml ($t_{0.05,\,73}$ = 5.70, P < 0.0001) while sediment-associated data provided a mean of 188,828 MPN/100 ml ($t_{0.05,\,73}$ = 5.51, P < 0.0001). A paired samples t-test confirmed *E. coli* concentrations were significantly higher in the sediments than in the overlying water column ($t_{0.05,\,73}$ = 5.53, P < 0.0001). Site averages for sediment-associated *E. coli* varied by an order of magnitude and remained in excess of 37,103 MPN/100 ml for all samples collected. Although this research is unable to show bacterial regrowth within deposited sediments, they confirm an abundant reservoir of *E. coli* available for resuspension given the necessary physical conditions.

DISCUSSION

This study was designed to evaluate man-made hydrologic systems routinely installed throughout the United States to control construction-derived runoff. Sediment basins are poorly understood from an ecological perspective, and systematic research has not been published regarding their potential for bacterial contamination or loading. Results generated here, however, are largely consistent with previous findings from both natural and created environments, affirming that *E. coli* are broadly distributed within both temperate soil and aquatic ecosystems (2, 3, 4, 6, 28, 29).

E. coli are present at significant densities in observed construction-derived runoff. Counts were significantly higher than EPA recommended water quality criteria but regression analysis of basin inlet data failed to find a significant predictor variable among those evaluated. Though counts measured from site runoff fluctuated considerably, results are consistent with similar research showing soil-associated bacterial distribution in natural ecosystems is highly variable and subject to a number of terrestrial physical and chemical influences (22, 32). The anthropogenic influence of construction-related soil compaction, grading and infill likely compound the difficulty in estimating associated bacterial densities.

E. coli levels are strongly influenced by rainfall depth. Other research suggests there can be significant differences between wet weather bacterial counts and those measured during dry-weather or base flow conditions (2, 6, 21, 29). Significantly lower *E. coli* densities under dry-weather conditions corresponded to lower observed concentrations of suspended solids within the water column. One of the established criteria for dry weather conditions was defined as days since last rainfall being greater than 1 day. It seems reasonable to suggest increased settling time allows bacteria adsorbed to suspended sediments further depositional opportunity, thus lowering *E. coli* density within the water column. The fact that no inlet or outlet data exist for dates where rainfall depth falls below 0.5 cm is further confirmation that precipitation is perhaps the most important physical influence within these sediment basins.

E coli are present and abundant within evaluated detention basins. The paucity of published ecological research relating specifically to construction site sediment basins has been discussed. Nevertheless in similar settings, enteric bacteria have been shown to persist under wide-ranging physical, chemical and biological conditions (7, 17, 26). Under storm conditions, *E. coli* densities from all evaluated sample locations significantly exceeded federally recommended concentrations to protect the swimming public from gastrointestinal illness.

Basin-specific findings were consistent with previously published research regarding bacterial concentrations and distribution in natural pond or lake systems (2, 3, 6, 7, 14, 17). *E. coli* correlations with suspended solids, temperature, pH, and conductivity were significant, but were only moderately predictive in terms of estimating bacterial density (r^2 = 0.42). The confounding nature of these results may corroborate previous findings that *E. coli* can survive and persist within temperate climates under wide ranging environmental conditions (18, 30). Strong intercorrelations among several independent variables (Table 3.2) may also be dampening model precision.

Deposited basin sediments are serving as consistent reservoirs of viable enteric bacteria. While *E. coli* was present in substantial numbers within the water column, the sediment-associated densities were 2 orders of magnitude greater across all dates and sites. Sediment-associated *E. coli* densities varied considerably among ponds and across dates and the overall mean exceeded 150,000 MPN/100 ml. Further, in weekly and event based sampling (n = 73), sediment-associated concentration fell below 1,000 MPN/100 ml on only 3 occasions. Burton et al (3) showed *E. coli* can persist longer than many other notable bacterial species within deposited sediments and that survival increases in organically-rich clay substrates. Though the origin and amount of any fill material utilized on evaluated construction sites was not determined, native soils are of the Cecil series, which have a typical clay content ranging between 5 – 35% (27) making them highly suitable to sustain populations of viable *E. coli*.

Resuspension of subsequent transport of mobilized bottom sediments during storm events help account for the persistently elevated *E. coli* levels in sediment basin discharge. Despite design advances to sediment basins in general, research has consistently confirmed that some amount of suspended material will be lost through the outlet (9, 24). Using experimental sediment basins, Jarrett (15) determined that 24% of cumulative sediment discharged from experimental basins resulted directly from resuspension of previously deposited material. With such a large reservoir of viable *E. coli* associated with bottom sediments, remobilization and exportation of resuspended material during rainfall events seems likely. This finding may help account for results showing a significant difference between *E. coli* densities found in construction site runoff and those observed in basin discharge.

Bacterial source tracking was beyond the scope of this project. Qualitative evidence of possible fecal sources however, was routinely observed and photographically documented. In addition to suspected soil-related bacterial sources, various feces deposited directly within basin catchments were visually confirmed. Animal tracks both entering and exiting areas subject to inundation were also detected on a regular basis. Extreme drought conditions during the course of research could potentially be attracting suburban wildlife to these newly created impoundments. In addition, 3 sites were hydro-seeded either during, or previous to the onset of sampling. Aside from grass seed and surfactant, these mixtures often contain “proprietary” blends of fertilizer, including manure.

Direct fecal input however, does not appear to sufficiently account for the observed difference in bacterial density between bottom sediments and those of the overlying water column or corresponding basin discharge. While additional research should undertake a mass balance accounting of water and sediments, it appears *E. coli* persists under observed environmental conditions found within these basins. Some have suggested there is limited practicality in relating *E. coli* quantities originating from hydromodified catchments to those established as criteria for contact recreation (4). However, because of the existing regulatory context of construction site sediment basins, these systems may provide an opportunity to further examine *E. coli* distribution at sites that in many ways define the nexus of point and nonpoint sources.

The significance of these cumulative findings may be considerable given the newly proposed Construction and Development Effluent Limitation Guidelines promulgated in November of 2008 by EPA as part of the revised National Pollutant Discharge Elimination System construction general permit (26). Because sediment basins have been shown by this research to contribute significant bacterial loading to downstream receiving waterbodies, resulting regulatory ramifications may be expected. Current modeling efforts to support development of total maximum daily loads do not account for bacteria allocations associated with construction activities. Yet these land-disturbing activities have physical impacts that occur at multiple spatial and temporal scales (8, 31) and *E. coli* persists under a multitude of environmental conditions (3, 12).

In summary, sediment basins studied during the course of this research adversely impacted the quality of downstream receiving waters in the Piedmont of South Carolina. *E. coli* appear to be viable, abundant and mobile within those systems monitored. Significantly disturbed and physically altered soils associated with construction site runoff nevertheless still correlate with elevated bacterial densities. Further, construction site sediment basins appear to be serving as reservoirs of viable *E. coli;* and resuspension of associated bottom sediments during storm events help account for the persistently elevated *E. coli* levels in site discharge.

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CHAPTER FOUR

ESCHERICHIA COLI DYNAMICS IN A GROUNDWATER INFLUENCED CONSTRUCTION SITE SEDIMENT BASIN

ABSTRACT

A groundwater-influenced wet sediment detention basin was monitored during nine months of 2008 to assess presence and distribution of *Escherichia coli* associated with construction site runoff and to evaluate whether the system may be acting as a source, sink or reservoir for enteric bacteria. Concentrations of *E. coli* were measured in water column, deposited sediments, and basin discharge. Results were analyzed collectively for all samples by location, as well as for storm (rainfall depth > 0.5 cm) and dry-weather conditions (rainfall depth < 0.5 cm; days since last rainfall > 1). Mean *E. coli* counts for water column (393 MPN/100 ml), outlet (272 MPN/100 ml) and sediments (117,591 MPN/100 ml) across all dates were highly variable. Pearson correlations between *E. coli* density and measured variables differed by sample location, but was highest for dry-weather TSS measured from the site outlet ($r = 0.87$; $p < 0.0001$). T-tests indicated mean *E. coli* densities in outlet discharge were significantly higher under storm conditions than during dry-weather conditions (t-stat = 2.74; $p = 0.0336$). Correspondingly, basin water column data showed storm *E. coli* counts

(mean = 995 MPN/100 ml; n = 7) also significantly exceeded those collected during dry-weather conditions (mean = 176 MPN/100 ml; n = 20) with a t-stat = 2.41; p = 0.0470. Both water column and outlet discharge *E. coli* densities exceeded recommended EPA water quality criteria under defined storm conditions. Multiple regression analysis found strong predictive capacity for *E. coli* density among the measured variables during dry weather conditions for both the outlet (r^2 = 0.76, $F_{3, 19}$ = 22.04, $p < 0.0001$) and the water column (r^2 = 0.59, $F_{7, 19}$ = 3.10, p = 0.0414). Aggregated results suggest further study is needed of these newly impounded hydrologic systems as construction sites are not presently included as part of routine regulatory modeling for bacterial wasteload allocation.

INTRODUCTION

Sediment detention basins are impoundments specifically designed and engineered to receive and temporarily hold construction site runoff. Primary benefits include dampening peak discharge rates and mitigating downstream impacts of eroded sediment. Site conditions and/or design requirements will largely dictate whether a sediment basin will ultimately be wet or dry. A wet detention basin is defined as an excavated area consisting of a permanent pool of water into which runoff is directed and detained until it is displaced during the next storm event, thus controlling both water quantity and quality (Mallin *et al*., 2002).

Dry basins on the other hand, are designed to empty completely within an allotted period of time. They are characterized by a primary outlet invert that is level, or flush with the bottom of the basin, thus facilitating discharge between runoff events (SC DHEC, 2005). In general, the size of the invert and configuration of any additional perforations in the outlet riser will control detention time for most runoff events. Detention time and basin volume are the two most important factors in terms of sediment removal within dry ponds (Shammaa *et al*., 2002).

From a regulatory perspective in South Carolina, construction projects of a sufficient size (typically greater than 4 hectares) must build and maintain dry sediment detention basins (Fig 4.1). This is largely a de facto requirement since the Construction General Permit does not contain specific language describing a dry detention basin yet primary outlet risers must include a minimum 6-inch diameter low flow orifice at the design depth (SC DHEC, 2006).

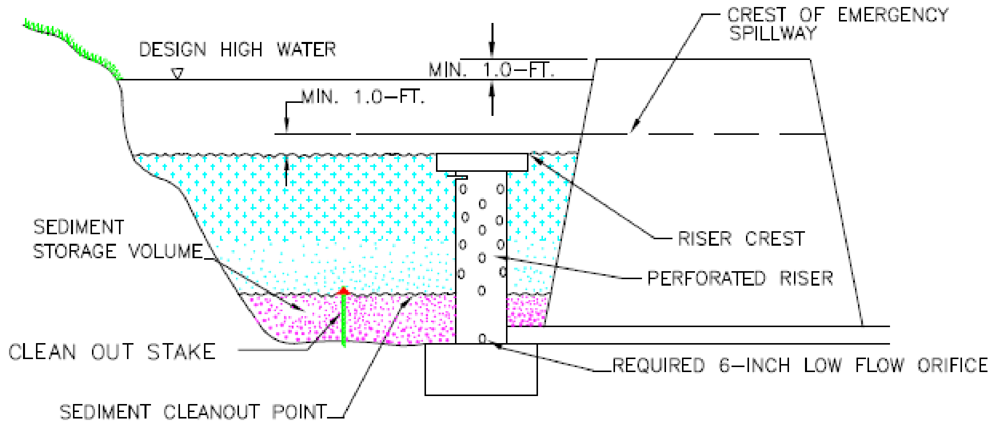

Figure 4.1 - Sediment basin standard drawing detail from the SC Stormwater Management BMP Manual (SC DHEC, 2005). The required low flow orifice for the primary outlet riser is consistent with establishment of a dry detention basin.

There are however, at least two site conditions for which a sediment basin designed to be “dry” would retain a permanent pool: 1) if the groundwater table is shallow enough to prohibit basin excavation to the design depth; and 2) if the basin is excavated to a sufficient depth to intercept existing groundwater flow. In either case the pond will likely exhibit more characteristics of a wet basin that those of a dry basin.

METHODS

Of the basins sampled as part of the research described in Chapters 2 and 3, the CC pond was considerably different than all others from both a design and hydrologic perspective. Original excavation occurred to an elevation or depth which clearly supported significant groundwater influence. To demonstrate this condition the CC site continued to maintain a consistent depth and regular discharge while all other basins were completely empty during one 40-day period without precipitation.

Groundwater has been shown to contain negligible quantities of *E. coli* (Byappanahalli *et al*., 2003). Since the CC pond was clearly inserting a hydrologic bias into results by diluting the water column and subsequent basin discharge, CC data were omitted from primary analysis contained in previous chapters. In order to remain consistent from a scientific perspective, those data were isolated and subjected to further statistical scrutiny in this chapter. Materials, methods and comprehensive site description details including basin location, size, drainage area, native soils, and photographs are included in Chapter 1. Results and corresponding analysis are provided below. Independent samples t-tests were used to determine whether the mean *E. coli* densities of storm and dry-weather conditions were significantly different from each other. Storm data were defined as those samples collected during a date in which rainfall depth exceeded 0.5 cm. Conversely, dry-weather data were defined as samples that were collected on a date where either no rainfall occurred or runoff did not enter the sediment basin. Identified differences between storm and dry-weather conditions for water column and outlet data were examined using independent samples t-tests, bivariate correlations and multiple regression. Statistical calculations were performed using SAS 9.2 and Microsoft Excel 2007. The statistical significance level was set at an alpha value of ≤ 0.05 unless otherwise stated.

RESULTS

Quantification of Rainfall-Related Differences

Simple means testing showed there was a numeric difference in *E. coli* densities between samples collected during or immediately after storm events and those collected during dry-weather conditions. Data for measured variables are provided in Table 4.1 for all dates as well as storm and dry-weather conditions. Log-transformed side-by-side comparison data are also displayed visually in Figures 4.2 – 4.4.

TABLE 4.1 - Means analysis for CC site sediment basin

Site	Sample Location	n	*E. coli* Density (MPN/100 ml)	TSS (mg/l)	Rainfall (cm)	DSLR[a] (days)	Temp (℃)	pH	DO (mg/l)	Cond (µS/cm)
CC	WC	27	393 +/- 643	30.1	0.66	6.95	27.6	6.4	6.4	89.9
	Outlet	28	272 +/- 534	26.8	0.66	7.05				
	Sediment	28	117,591 +/- 116,420		0.66	7.05				
CC	WC	7	995 +/- 866	63.4	2.40	0.29	26.9	5.9	5.3	70.9
Storm	Outlet	7	895 +/- 807	60.4	2.40	0.29				
(R > 0.5 cm)	Sediment	7	37,540 +/- 20,609		2.40	0.29				
CC	WC	20	176 +/- 409	18.8	0.00	11.21	27.6	6.5	6.5	94.7
Dry-Weather	Outlet	20	58 +/- 84	13.1	0.00	11.21				
(R < 0.5 cm)	Sediment	20	150,925 +/- 472,130		0.00	11.21				

Measured Variables: *E. coli* Density through Cond.

E. coli density values shown as MPN/100 ml +/- standard deviation
[a] Days since last rainfall; *n* , number of samples

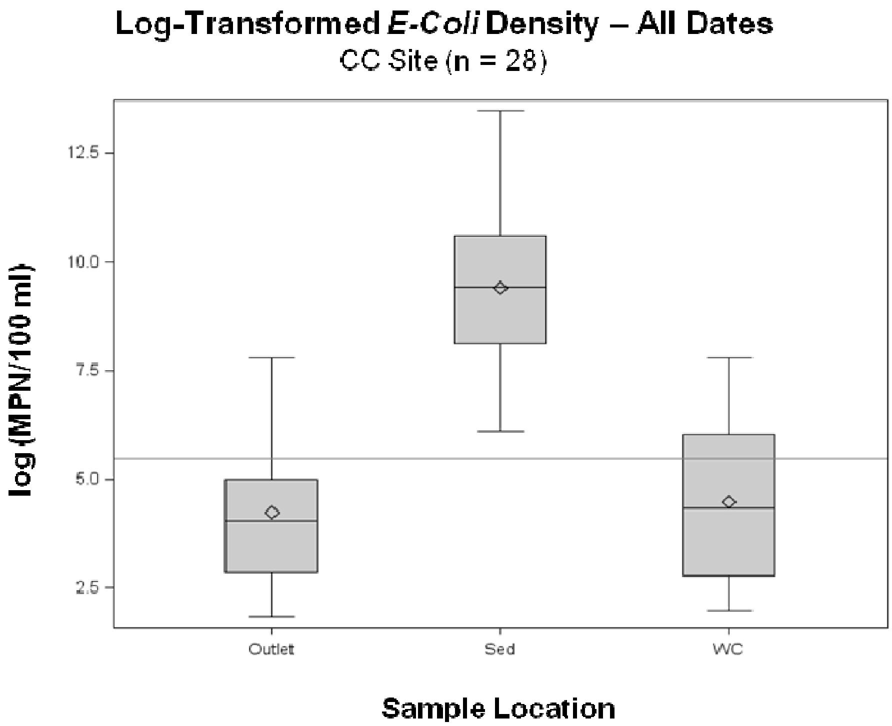

Figure 4.2 - Box and whisker plot of mean E. coli densities at the CC site for all dates by sample location. Reference line is drawn at EPA water quality criteria for contact recreation

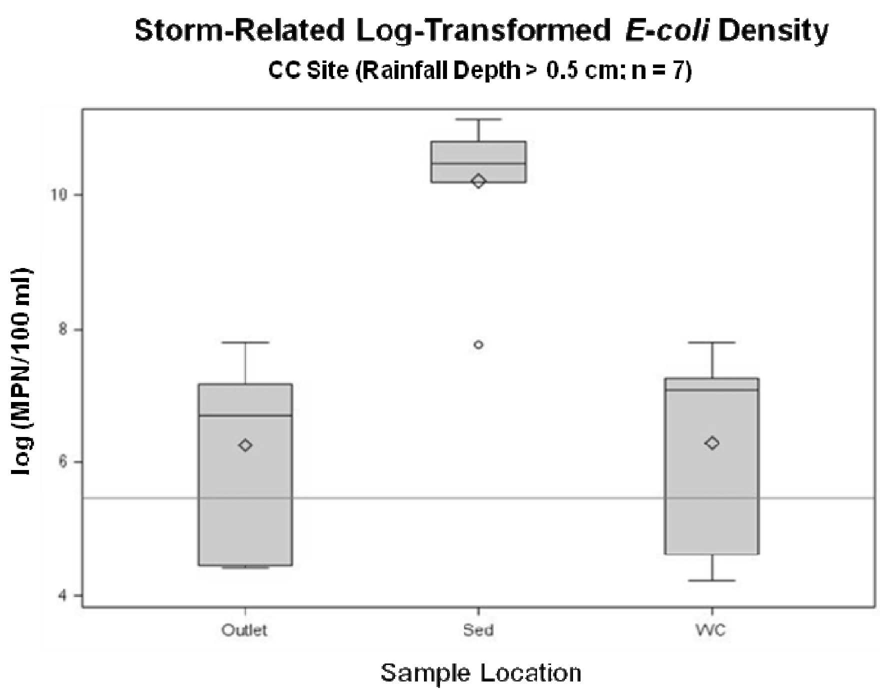

Figure 4.3 - Box and whisker plot of mean E. coli densities at the CC site for storm-related dates by sample location. Reference line is drawn at EPA water quality criteria for contact recreation

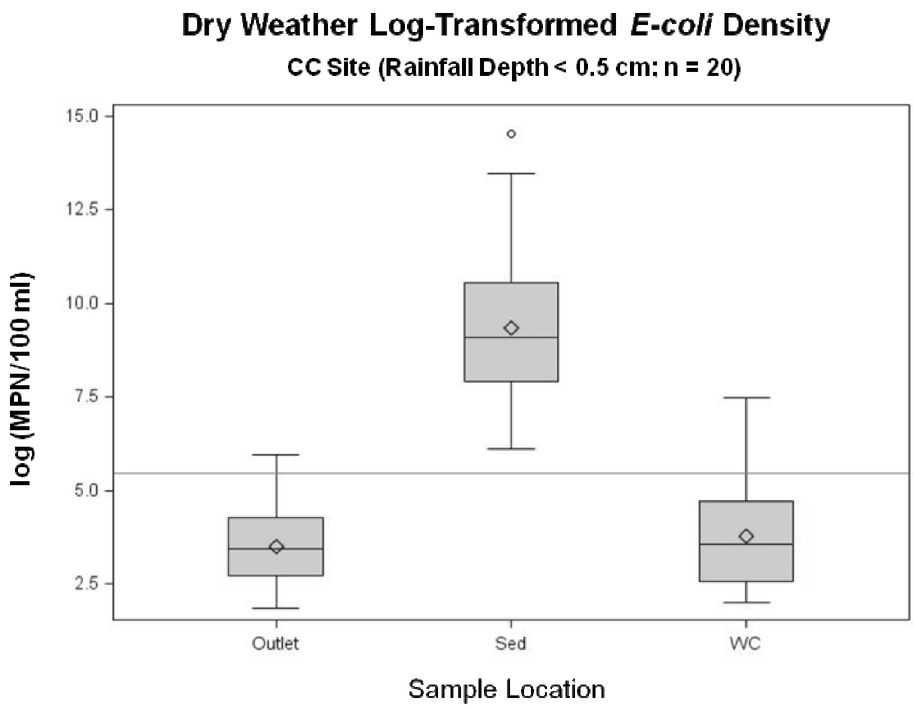

Figure 4.4 - Box and whisker plot of mean E. coli densities at the CC site for dry-weather conditions by sample location. Reference line is drawn at EPA water quality criteria for contact recreation

Results from the CC outlet indicated that storm *E. coli* densities (mean = 895 MPN/100 ml; n = 7) were significantly higher than those collected during dry-weather (mean = 58 MPN/100 ml, n = 20) conditions (t-stat = 2.74; p = 0.0336). Within the water column, storm *E. coli* counts (mean = 995 MPN/100 ml; n = 7) significantly exceeded those collected during dry-weather conditions (mean = 176 MPN/100 ml; n = 20) with a t-stat = 2.41; p = 0.0470.

For sediment samples however, results failed to find a significant difference between the means for storm (mean = 37,540 MPN/100 ml; n = 7) and dry-weather conditions (mean = 150,925 MPN/100 ml; n = 20). The equality of variance test suggested a strong likelihood of unequal variances for sediment

MPN, and t-test results confirmed no significant difference (t-stat = 1.07; p = 0.2974).

Outlet Results

T-test results indicated the true mean for all outlet samples (mean = 272 MPN/100 ml; t-stat = 0.37; p = 0.3575; n = 27) was not significantly different than the EPA recommended criterion of 235. Since previous results suggested a significant difference between storm and dry-weather conditions as measured from the outlet, those data were separated for further consideration.

Storm-related outlet *E. coli* densities were found to be significantly higher than the EPA recommended standard (mean = 895; t-stat = 2.16; p = 0.0739/2). Conversely, corresponding analysis suggested the recommended criterion of 235 was significantly larger than the mean CC outlet MPN for dry weather conditions (mean = 58 MPN/100 ml; t-stat = -9.41; p< 0.0001). The CC site storm outlet mean thus compares more closely with the outlet mean across all other sites and dates (1368 MPN/100 ml) than does the mean of the CC dry weather dates.

Pearson analysis for all outlet data revealed that only TSS (r =0.44; p = 0.0196) and rainfall depth (r = 0.45; p = 0.0166) were moderately correlated with MPN. Multiple regression yielded a weak r-square value (0.28) that was barely significant ($F_{3, 27}$ = 3.09, p = 0.0462). As above, data were separated for dry-weather and storm conditions.

Storm data predictor variables offered no significant correlation with *E. coli* density. Multiple regression analysis failed to find a significant linear model that predicts *E. coli* density from any of the measured independent variables. The moderately strong coefficient of determination ($r^2 = 0.67$) corresponded to indeterminate results of the significance test for the regression line equation ($F_{3,\,7} = 2.03$, $p = 0.2880$). Thus for storm outlet data collected from the CC site there fails to be a statistically reliable prediction model for suspended solids, rainfall depth and DSLR.

Dry-weather CC outlet results indicated there was one significant Pearson correlation among the measured variables. TSS displayed a strong positive relationship with *E. coli* densities ($r = 0.87$; $p < 0.0001$). Multiple regression of the observed independent variables with MPN found a strong relationship ($r^2 = 0.76$, $F_{3,\,20} = 22.04$, $p < 0.0001$). Results suggest that under dry-weather conditions, TSS would be important in terms of prediction within the CC outlet regression model.

Bottom Sediment Results

T-test results indicated the true mean for CC sediment samples was significantly greater than 5.86, which is the log-transformed value of the EPA criterion 235 (mean = 9.57; t-stat = 10.41; $p < 0.0001/2$; n = 28). *E. coli* density was not significantly correlated with either rainfall depth or DSLR. In fact, multiple regression yielded a very weak coefficient of determination ($r^2 = 0.04$)

and corresponded to indeterminate results of the significance test for the regression line equation ($F_{2, 28}$ = 0.58, p = 0.5673).

Water Column Results

T-tests indicated the mean for all CC water column samples was not significantly greater than recommended water quality criteria (mean = 393 MPN/100 ml; t-stat = 1.30; p = .2046/2; n = 27). Storm-related mean water column *E. coli* densities were found to be significantly higher than the EPA recommended standard (mean = 995 MPN/100 ml; t-stat = 2.32; p = 0.0593/2) and were comparable with water column means found across all other sites (877 MPN/100 ml). Analysis of mean dry-weather water column counts from the CC basin were indeterminate (mean = 176 MPN/100 ml; t-stat = -0.64; p = 0.5275) and indicated there was not enough evidence to confirm a difference with the 235 criterion.

Bivariate correlations for all dates revealed three predictor variables were related to *E. coli* density: TSS (r = 0.35, p = 0.0638), rainfall (r = 0.35, p = 0.0709), pH (r = -0.32, p = 0.0968), though none at the conventional alpha (α = 0.05). *E. coli* densities regressed against all measured variables resulted in a model that was only moderately predictive (r^2 = 0.41, $F_{7, 26}$ = 2.92, p = 0.0375).

For storm conditions, predictor variables offered no significant correlation with *E. coli* density within the water column. Multiple regression analysis failed to find a linear model that adequately predicted *E. coli* density from any of the measured independent variables. The weak coefficient of determination (r^2 = 0.17) corresponded to indeterminate results of the significance test for the regression line equation ($F_{5, 7}$ = 0.83, p = 0.4149).

For dry-weather CC water column data, there was one significant Pearson correlation among the measured variables and MPN. Conductivity displayed a moderate but significant positive relationship with *E. coli* densities (r = 0.59; p = 0.0061). Multiple regression of the observed independent variables with MPN found a moderately strong correlation (r^2 = 0.59, $F_{7, 19}$ = 3.10, p = 0.0414). As with outlet data, dry-weather conditions provided improved predictive modeling for the CC water column.

CONCLUSIONS

E. coli levels associated with the CC basin appear to be strongly influenced by rainfall depth. Other research suggests there can be significant differences between wet weather bacterial counts and those measured during dry-weather or base flow conditions (Whitman *et al*., 2006; An *et al*., 2002; Davies and Bavor, 2000). Lower *E. coli* density under dry-weather conditions corresponded to lower concentrations of suspended solids (Table 4.1) in both basin discharge and water column. One of the established criteria for dry weather conditions was defined as days since last rainfall being greater than 1 day. It seems reasonable to suggest increased settling time may allow bacteria adsorbed to suspended sediments further depositional opportunity, thus lowering *E. coli* density within the water column, and ultimately basin discharge.

Further, Byappanahalli *et al.*, (2003) showed in temperate regions that groundwater contained negligible quantities of *E. coli*. Lower bacterial concentrations obtained during dry-weather conditions are also likely to result from dilution by groundwater, which sustains the site's permanent pool.

E coli are present and abundant within the CC wet detention basin. The paucity of published ecological research relating specifically to construction site sediment basins has been discussed in previous chapters. Nevertheless in similar settings, enteric bacteria have been shown to persist under wide-ranging physical, chemical and biological conditions (Davis *et al*., 2005; Whitman *et al*., 2004; LaLiberte and Grimes, 1981). Under storm conditions, *E. coli* densities in all evaluated sample locations significantly exceeded federally recommended concentrations to protect the swimming public from gastrointestinal illness.

Deposited sediments in the CC wet basin are serving as a consistent reservoir of viable enteric bacteria. While sediment-associated *E. coli* densities varied considerably during the course of research, the overall mean exceeded 100,000 MPN/100 ml. Further, in weekly and event based sampling (n = 28), sediment-associated concentration fell below 1235 MPN/100 ml on only 2 occasions (Appendix A). Burton *et al*. (1986) showed that *E. coli* can persist longer than other evaluated bacterial species within deposited sediments and that survival time increases in organically-rich clay substrates. Though the origin and amount of fill material brought to the CC construction site was not determined, native soils are of the Cecil series, which have a typical clay content ranging between 5 – 35% (USDA, 1993) making them highly suitable to sustain populations of viable *E. coli*.

Despite design advances to sediment basins in general, research has confirmed that some amount of suspended material will be lost through the outlet (Thaxton and McLaughlin, 2004; Fennessey and Jarrett, 1996). Using experimental sediment basins, Jarrett (2001) determined that 24% of cumulative sediment discharged from experimental basins was a direct result of resuspension of previously deposited material. With such a large reservoir of viable *E. coli* associated with bottom sediments, remobilization and exportation of resuspended material during rainfall events seems likely.

The CC basin is contributing to bacterial loading of its receiving waters. Outlet bacterial concentrations ranged measurably, but always contained some presence of *E. coli* in every sample (n = 28; Appendix A). It has been established that the CC basin exhibits consistent discharge, even under dry-weather conditions when *E. coli* densities as measured from the site outlet are lowest. Since the basin was excavated as a direct result of construction-related activities and would not otherwise have been built and consistently discharging effluent containing *E. coli*, it is acting as a net source of viable bacteria.

Measured variables offer only limited predictive capacity for estimating *E. coli* density. While regression analysis provides relatively strong models for understanding *E. coli* density at outlet and water column sample locations under dry weather conditions, those circumstances do not reflect the rainfall-influenced bacterial levels that would likely represent the largest threat to human health. Consistent results across multiple dates and under multiple environmental conditions suggest a considerable need for additional and corroborating research.

This study was designed to evaluate man-made hydrologic systems routinely installed throughout the United States to control construction-derived sediments. The significance of these cumulative findings beyond potential impacts to human health may be substantial given the newly proposed

Construction and Development Effluent Limitation Guidelines promulgated in November of 2008 by EPA as part of the revised National Pollutant Discharge Elimination System construction general permit (EPA, 2008). Should sediment basins be found to contribute a significant amount of bacterial loading to downstream receiving waterbodies, resulting regulatory strengthening could be extensive.

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CHAPTER FIVE

SUMMARY CONCLUSIONS

New construction serves an integral role in meeting the Nation's evolving need for growth. Based on emerging requirements of an aging population, demographic shifts and increased immigration, it is estimated that 35 million acres of previously unbuilt land will be developed before 2030 in the United States (Nelson, 2004). Such demand emphasizes the need for understanding the full range of environmental impacts related to construction activities. While the effects of sedimentation have been well documented (Ehrhart *et al*., 2002; Wood and Armitage, 1997), the possibility of construction-derived microbial contamination has remained scientifically unexplored.

This study was undertaken to evaluate *E. coli* contributions from man-made hydrologic systems routinely installed to control construction site runoff. Results generated through this research are largely consistent with previous findings from both natural and created environments, affirming that *E. coli* are broadly distributed within both temperate soil and aquatic ecosystems (Whitman *et al*., 2006; Davis *et al.*, 2005; Whitman *et al*., 2004; Byappanahalli *et al*., 2003; An *et al*., 2002; Davies and Bavor, 2000; Burton *et al*., 1986). While recent research has concluded *E. coli* can persist for extended periods outside of a host or primary environment (Ishii and Sadowsky, 2008; Winfield and Groisman, 2003;

Gordon *et al*., 2002), until now these findings have not been reconciled with construction related activities.

FINDINGS

***E. coli* were present at significant concentrations in observed construction site runoff.** Across all sites and dates, mean *E. coli* densities measured from basin inlets were significantly higher than EPA recommended water quality criteria. Though counts measured from site runoff fluctuated considerably, results are consistent with similar research showing soil-associated bacterial distribution in natural ecosystems is highly variable and subject to a number of terrestrial physical and chemical influences (Wu *et al*., 2009, Nunan *et al*., 2001).

Construction site sediment basins were serving as consistent reservoirs of viable *E. coli*. Mean water column bacteria counts were significantly higher than EPA's recommended standard across sites and dates. In similar lentic settings, enteric bacteria have been shown to persist under wide-ranging environmental conditions (Davis *et al.*, 2005; Whitman *et al*., 2004; LaLiberte and Grimes, 1981). Pearson analysis indicated that within the water column there were 5 independent variables significantly correlated with *E. coli* density: TSS, temperature, pH, DSLR and conductivity, though none above a 0.50 value.

While sediment-associated *E. coli* densities also varied considerably during the course of research, the overall mean exceeded 180,000 MPN/100 ml. Burton *et al.* (1986) showed *E. coli* can persist within deposited sediments and that survival time increases in organically-rich clay substrates. Though compositional analysis was not conducted as part of this study, native soils at all sites were comprised of a proportionally high amount of clay, making them highly suitable to sustain populations of viable *E. coli*.

E. coli levels in all basins were strongly influenced by rainfall. Existing research suggests there can be significant differences between wet weather bacterial counts and those measured during dry-weather or base flow conditions (Whitman *et al.*, 2006; An *et al*., 2002; Davies and Bavor, 2000). Results from this project confirmed mean *E. coli* counts collected during storm events from basin outlets and water columns were significantly higher than those found at the same sample locations under dry weather conditions. These findings suggest increased settling time may allow bacteria adsorbed to suspended sediments further depositional opportunity, thus lowering *E. coli* density within the water column, and ultimately basin discharge. Results appear to confirm that rainfall depth is the principle physical influence within these sediment basins. Further, if no precipitation fell during the course of construction activities, there would be only limited risk of bacterial contamination to receiving waterbodies.

Sediment basins were contributing to net bacterial loading of receiving waters. For dry detention basins, *E coli* concentrations measured from basin outlets were significantly higher than corresponding inlet concentrations. With such a large reservoir of viable *E. coli* associated with bottom sediments (mean = 188,000 MPN/100 ml), remobilization and subsequent exportation of resuspended material during rainfall events seems likely.

The CC wet detention basin was excavated as a direct result of construction-related activities and to address permit requirements related to attenuating peak discharges. Installation of the CC basin created a new man-made hydrologic feature with a permanent pool where one did not exist previously. Interception of groundwater flow within the basin provides consistently discharging effluent containing *E. coli*, and thus CC is also acting as a net source of viable bacteria to receiving waters.

Regression modeling had limited predictive capacity using measured independent variables. Measured variables offered only limited predictive capacity for estimating *E. coli* density. Pearson correlations with suspended solids, temperature, pH, and conductivity were significant within water columns, but were only moderately useful in terms of estimating bacterial density. These results may confirm previous findings that *E. coli* can survive and persist within temperate climates under wide ranging environmental conditions. It was only for dry weather conditions (rainfall < 0.5 cm) when DSLR was set to greater than 1 day that regression modeling yielded relatively high r^2 values (0.76 at outlet; 0.59 for water column). It seems likely these higher values result from stronger correlations with TSS brought about by the absence of runoff-related turbulence and correspondingly increased settling times.

Current state and federal regulatory environments underscore the necessity for additional research. Current regulatory modeling efforts to support development of total maximum daily loads do not account for bacteria wasteload allocations associated with construction activities. Yet these land-disturbing activities have physical impacts that occur at multiple spatial and temporal scales. Further, the significance of these cumulative findings beyond potential risks to human health may be considerable given the newly proposed Construction and Development Effluent Limitation Guidelines (EPA, 2008). These guidelines and presumptive changes to relevant federal and state regulations are likely to affect permitted construction activities well into the future. Sediment basins are principle design components of almost all medium to large construction sites. Should sediment basins be found to contribute significant bacterial loading to receiving waterbodies, resulting regulatory ramifications could be extensive. Consistent results indicating the possibility of such loading across multiple dates and under multiple environmental conditions suggest a demonstrated need for additional and corroborating research.

DESIGN CONSIDERATIONS

1. To dissipate energy from inlet pipe(s) have new basins incorporate a sediment forebay. It has been shown that sediment basins are serving as reservoirs of viable *E. coli* and that these bacteria are subject to resuspension and exportation resulting from the energy associated with incoming stormwater. A forebay would isolate such turbulence and discharge into the remaining basin area through a berm of washed stone.

2. Beyond the washed stone berm, water would enter a series of serpentine woven geotextile fabric baffles. Such baffles would increase the residence time of sediment-laden water and allow further opportunity for deposition of suspended material before reaching the basin outlet structure. Energy of water flowing out of the forebay would also be further dampened.

3. Consider use of hydroseeded vegetation within the bottom portions of the basin in addition to the side slopes. In addition to binding the substrate and minimizing resuspension, such vegetation would provide the opportunity for more microbial activity, perhaps attracting heterotrophic species that graze on *E. coli*. Rooted vegetation could also remove moisture from the top layer of the substrate, making it more difficult for *E. coli* to remain viable.

4. Require that basin inlet pipes be as far away as possible from primary basin outlet structures. Not only would such a design consideration address the effects of inlet-related energy on basin discharge, but it would also minimize the opportunity of “short-circuiting.”

FUTURE DIRECTIONS

1. To evaluate how bacterial density responds to changes in environmental conditions, construct scale model(s) of sediment basin to undertake controlled experimentation.
2. To quantify bacterial loading from active construction sites as well as sediment basins, collect and analyze detailed flow data for construction of hydrographs and chemographs.
3. To confirm preferential affinity for smaller sediment size, analyze eroded particle size distribution from construction sites and particle size distribution for basin water column and outlets.
4. To address spatial variability, collect storm data from basins in other physiographic provinces (Sandhills, coastal plan, coast, etc). This can and should be conducted with full cooperation from regional stormwater managers.
5. Quantify and index existence of heterotrophic bacteria in the basins whose presence might inhibit viability of *E. coli* through competition for nutrients and/or grazing.
6. In order to strengthen regression modeling, measure concentrations of bio-available nitrogen and phosphorous, particularly in sediments.
7. Determine and quantify pathogenicity of *E. coli* strains found in sediment basin. Genetic level microbiologist will be required to address this level of analysis.
8. Solidify laboratory procedure for bacterial desorption from sediments such as chemical agents (phosphate buffered saline), sonication, vortexing, etc. Most sediment-related densities in published literature have units of counts/100 g of dry weight.
9. Investigate the ecology of naturalized *E. coli* strains to determine: why they survive and grow better in the environment (Ishii *et al*., 2008) and what phenotypic mechanisms allow them to persist in soils.

10. Examine interaction between bacteria and the anionic polymer, polyacrylamide (PAM). PAM is associated with construction activities because of its ability to flocculate suspended sediments in water but there is only limited research on biological interactions or fate in the environment.

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APPENDIX

COMPREHENSIVE POND DATA TABLE

	Date	Site	Sample Location	*E. coli* Density (MPN/100ml)	TSS (mg/l)	Cond (µS/cm)	DO (mg/l)	pH	Temp (℃)	DSLR (days)	Rainfall (in)
1	7/8/2008	AHC	WC	5	423.7					0.0	0.3
2	7/11/2008	AHC	WC	1	57.2	142.4		5.8	26.0	3.0	0.0
3	7/22/2008	AHC	WC	56	80.4	194.2	4.7	6.3	23.2	0.5	1.0
4	7/25/2008	AHC	WC	16	15.5	163.1	5.9	7.0	26.4	3.5	0.0
5	7/27/2008	AHC	WC	110	121.1	218.3	5.4	7.3	29.7	0.5	0.7
6	7/30/2008	AHC	WC	11	12.2	210.0	5.9	6.6	27.7	3.5	0.0
7	8/1/2008	AHC	WC	1	7.2	319.6	5.5	6.1	26.0	5.5	0.0
8	8/13/2008	AHC	WC	28	185.0	88.6	5.0	5.9	24.8	0.0	0.4
9	8/15/2008	AHC	WC	3	14.4	212.9	5.0	6.5	27.5	2.0	0.0
10	8/23/2008	AHC	WC	51	25.8	390.5	10.4	7.3	28.6	10.0	0.0
11	8/25/2008	AHC	WC	1,203	269.2	90.2	5.6	5.9	26.2	0.0	0.7
12	8/26/2008	AHC	WC	1,986	129.1	54.3	5.9	5.9	22.5	0.0	1.6
13	8/29/2008	AHC	WC	64	46.5	82.4	5.3	6.1	28.9	1.0	2.4
14	9/26/2008	AHC	WC	416	100.4	131.3	8.0	5.7	20.6	0.0	0.6
15	5/15/2008	C81A	WC	308	35.9	46.9	2.7	6.4	25.0	3.0	0.0
16	5/16/2008	C81A	WC	260	47.7	46.6	5.3	6.2	28.8	0.5	0.4
17	5/17/2008	C81A	WC	147	105.4	47.3	5.1	6.4	29.2	1.5	0.0
18	5/18/2008	C81A	WC	299	33.2	49.0	5.9	6.7	27.4	2.5	0.0
19	5/19/2008	C81A	WC	214	24.3	51.3	5.1	6.2	31.0	0.5	0.1
20	5/23/2008	C81A	WC	1	36.1	50.8	4.9	6.3	33.8	4.5	0.0
21	7/8/2008	C81A	WC	1,986	392.2					0.0	0.6
22	7/11/2008	C81A	WC	158	42.9	73.5	3.3	6.0	27.6	3.0	0.0
23	7/19/2008	C81A	WC	82	53.3	178.1	3.9	6.1	35.5	10.0	0.0
24	7/22/2008	C81A	WC	219	266.3	58.0	2.9	5.6	22.8	0.5	0.4
25	7/25/2008	C81A	WC	46	74.6	71.3	6.1	5.9	27.3	3.5	0.0
26	7/27/2008	C81A	WC	250	83.8	57.9	5.8	5.8	32.7	0.5	0.7
27	7/30/2008	C81A	WC	173	30.7	57.2	6.3	5.7	31.2	3.5	0.0
28	8/1/2008	C81A	WC	43	21.2	68.4	4.9	6.0	30.1	5.5	0.0
29	8/13/2008	C81A	WC	1,046	153.8	51.5	5.1	5.2	25.4	0.0	0.6
30	8/15/2008	C81A	WC	167	46.5	46.6	4.6	5.7	32.8	2.0	0.0
31	8/23/2008	C81A	WC	980	9.1	76.4	4.8	5.2	31.2	10.0	0.0
32	8/25/2008	C81A	WC	2,420	237.6	45.3	5.5	5.2	25.8	0.0	1.1
33	8/26/2008	C81A	WC	1,414	192.6	25.8	7.0	5.5	22.6	0.0	1.4
34	8/29/2008	C81A	WC	548	18.2	29.8	5.2	5.5	31.6	1.0	2.3
35	9/26/2008	C81A	WC	2,420	41.9	55.9	6.4	5.2	22.0	0.0	0.5
36	7/8/2008	C81B	WC	2,420	23.7					0.0	0.6
37	7/11/2008	C81B	WC	1,300	136.6	122.9	7.2	6.0	30.1	3.0	0.0
38	8/13/2008	C81B	WC	1,203	51.6	11.8	3.2	4.5	25.6	0.0	0.6
39	8/25/2008	C81B	WC	517	23.8	40.8	4.7	5.4	26.9	0.0	1.1
40	8/26/2008	C81B	WC	1,046	140.6	15.6	6.1	5.4	22.9	0.0	1.4

	Date	Site	Sample Location	*E. coli* Density (MPN/100ml)	TSS (mg/l)	Cond (µS/cm)	DO (mg/l)	pH	Temp (℃)	DSLR (days)	Rainfall (in)
41	5/13/2008	CC	WC	11	22.9	69.7	5.5	6.1	22.3	1.0	0.0
42	5/15/2008	CC	WC	7	16.1	68.6	5.9	6.1	22.1	3.0	0.0
43	5/16/2008	CC	WC	69	22.3	71.1	6.3	6.1	23.6	0.5	0.4
44	5/17/2008	CC	WC	17	18.2	72.6	7.6	6.1	23.8	1.5	0.0
45	5/18/2008	CC	WC	8	10.2	73.1	2.4	6.3	22.6	2.5	0.0
46	5/19/2008	CC	WC	10	8.0	53.0	7.8	6.3	24.1	0.5	0.1
47	5/23/2008	CC	WC	122	12.8	79.0	8.6	6.4	24.6	4.5	0.0
48	5/30/2008	CC	WC	87	4.8	86.4	7.7	7.0	25.8	11.5	0.0
49	6/6/2008	CC	WC	128	9.4	103.0	9.4	8.1	31.0	18.5	0.0
50	6/13/2008	CC	WC	34	14.5	115.6	7.5	6.5	31.0	18.5	0.0
51	6/20/2008	CC	WC	1,733	9.2	138.9	4.9	6.3	29.5	25.5	0.0
52	6/27/2008	CC	WC	816	12.8	126.7	5.5	6.2	28.2	25.5	0.0
53	7/4/2008	CC	WC	16	18.0	118.3	6.9	7.0	27.5	32.5	0.0
54	7/8/2008	CC	WC	1,203	137.5					0.0	1.0
55	7/11/2008	CC	WC	37	37.6	90.7	7.2	6.2	29.5	3.0	0.0
56	7/19/2008	CC	WC	14	50.9	119.0	6.5	7.1	33.2	10.0	0.0
57	7/22/2008	CC	WC	345	84.7	90.7	3.2	5.2	27.0	0.5	0.7
58	7/25/2008	CC	WC	17	12.0	98.3	5.0	6.4	28.2	3.5	0.0
59	7/27/2008	CC	WC	517	22.1	107.2	11.2	6.6	30.5	0.5	0.2
60	7/30/2008	CC	WC	272	22.9	100.2	9.1	6.6	30.9	3.5	0.0
61	8/1/2008	CC	WC	44	21.2	102.3	5.7	6.5	32.0	5.5	0.0
62	8/8/2008	CC	WC	38	25.9	104.6	7.5	6.3	29.5	12.5	0.0
63	8/13/2008	CC	WC	1,414	42.0	82.1	6.3	6.1	27.2	0.0	0.8
64	8/15/2008	CC	WC	101	27.4	82.2	4.9	6.7	30.5	2.0	0.0
65	8/23/2008	CC	WC	12	20.9	93.2	5.2	6.3	27.2	10.0	0.0
66	8/25/2008	CC	WC	1,414	30.8	82.4	5.9	6.0	25.2	0.0	0.6
67	8/26/2008	CC	WC	2,420	59.6	57.9	5.1	5.7	24.5	0.0	1.1
68	8/29/2008	CC	WC	102	67.2	41.4	5.2	6.0	33.6	1.0	2.6
69	8/13/2008	CH1	WC	2,420	145.9	61.9	5.2	5.6	25.4	0.0	0.7
70	8/15/2008	CH1	WC	43	32.3	138.2	5.1	6.4	35.2	2.0	0.0
71	8/25/2008	CH1	WC	2,420	107.6	80.3	6.1	6.0	25.7	0.0	0.6
72	8/26/2008	CH1	WC	2,420	238.8	46.6	5.3	5.6	24.1	0.0	1.6
73	8/29/2008	CH1	WC	115	35.0	45.7	4.3	5.4	34.8	1.0	2.4
74	9/26/2008	CH1	WC	225	78.7	196.7	7.4	5.4	21.4	0.0	0.6
75	8/13/2008	CH2	WC	2,420	96.6	62.8	5.3	5.3	26.0	0.0	0.7
76	8/15/2008	CH2	WC	921	7.9	82.0	4.4	6.1	34.5	2.0	0.0
77	8/25/2008	CH2	WC	2,420	215.9	57.9	4.6	5.2	27.2	0.0	0.6
78	8/26/2008	CH2	WC	2,420	71.4	27.7	5.1	4.9	23.7	0.0	1.6
79	8/29/2008	CH2	WC	105	10.3	24.1	4.9	5.0	33.8	1.0	2.4
80	9/26/2008	CH2	WC	147	94.1	100.5	7.0	5.4	21.7	0.0	0.6
81	7/8/2008	RP	WC	2,420	199.2					0.0	0.3
82	7/11/2008	RP	WC	2,420	52.4	150.5	0.9	5.8	26.7	3.0	0.0
83	7/22/2008	RP	WC	1,553	116.1	51.9	1.2	5.5	22.8	0.5	1.0
84	7/25/2008	RP	WC	104	30.3	67.1	2.6	6.0	26.7	3.5	0.0
85	7/27/2008	RP	WC	107	58.6	47.9	5.1	6.0	30.5	0.5	0.7
86	7/30/2008	RP	WC	32	14.3	44.5	6.1	5.8	31.7	3.5	0.0
87	8/1/2008	RP	WC	148	15.0	49.7	4.7	5.7	31.7	5.5	0.0
88	8/13/2008	RP	WC	1,300	70.8	47.2	5.7	5.0	24.5	0.0	0.6
89	8/15/2008	RP	WC	17	18.0	46.1	5.3	5.6	32.1	2.0	0.0
90	8/25/2008	RP	WC	2,420	131.9	55.2	5.2	5.6	25.0	0.0	0.8

	Date	Site	Sample Location	*E. coli* Density (MPN/100ml)	TSS (mg/l)	Cond (µS/cm)	DO (mg/l)	pH	Temp (℃)	DSLR (days)	Rainfall (in)
91	8/26/2008	RP	WC	2,420	108.3	53.2	6.4	5.8	23.5	0.0	1.6
92	8/29/2008	RP	WC	80	13.4	42.1	5.1	6.0	30.4	1.0	2.7
93	5/30/2008	SC	WC	1,986	155.1	44.9	5.2	6.3	29.2	11.5	0.0
94	7/8/2008	SC	WC	2,420	200.4					0.0	0.4
95	7/22/2008	SC	WC	2,420	88.2	77.9	2.6	4.9	22.6	0.5	0.7
96	7/27/2008	SC	WC	344	126.7	53.5	4.0	5.2	29.5	0.5	0.7
97	8/13/2008	SC	WC	2,420	271.0	61.8	6.2	5.3	23.8	0.0	0.8
98	8/15/2008	SC	WC	308	46.9	90.4	3.9	5.9	30.5	2.0	0.0
99	8/25/2008	SC	WC	461	186.8	56.9	4.9	5.5	25.4	0.0	1.0
100	8/26/2008	SC	WC	866	41.0	52.2	5.7	5.4	22.5	0.0	1.5
101	8/29/2008	SC	WC	65	41.0	55.6	6.0	5.5	27.7	1.0	2.4
102	7/8/2008	AHC	Sed	4,747						0.0	0.3
103	7/11/2008	AHC	Sed	1						3.0	0.0
104	7/22/2008	AHC	Sed	13,281						0.5	1.0
105	7/25/2008	AHC	Sed	24,307						3.5	0.0
106	7/27/2008	AHC	Sed	9,681						0.5	0.7
107	7/30/2008	AHC	Sed	6,797						3.5	0.0
108	8/1/2008	AHC	Sed	11,000						5.5	0.0
109	8/13/2008	AHC	Sed	1,423						0.0	0.4
110	8/15/2008	AHC	Sed	74,241						2.0	0.0
111	8/23/2008	AHC	Sed	7,513						10.0	0.0
112	8/25/2008	AHC	Sed	10,496						0.0	0.7
113	8/26/2008	AHC	Sed	124,415						0.0	1.6
114	8/29/2008	AHC	Sed	210,380						1.0	2.4
115	9/26/2008	AHC	Sed	21,162						0.0	0.6
116	5/15/2008	C81A	Sed	3,290						3.0	0.0
117	5/16/2008	C81A	Sed	1,137						0.5	0.4
118	5/17/2008	C81A	Sed	2,342						1.5	0.0
119	5/18/2008	C81A	Sed	4,520						2.5	0.0
120	5/19/2008	C81A	Sed	10,202						0.5	0.1
121	5/23/2008	C81A	Sed	2,334						4.5	0.0
122	7/8/2008	C81A	Sed	76,633						0.0	0.6
123	7/11/2008	C81A	Sed	40,812						3.0	0.0
124	7/19/2008	C81A	Sed	16,345						10.0	0.0
125	7/22/2008	C81A	Sed	8,429						0.5	0.4
126	7/25/2008	C81A	Sed	5,760						3.5	0.0
127	7/27/2008	C81A	Sed	123,484						0.5	0.7
128	7/30/2008	C81A	Sed	281,897						3.5	0.0
129	8/1/2008	C81A	Sed	73,655						5.5	0.0
130	8/13/2008	C81A	Sed	13,697						0.0	0.6
131	8/15/2008	C81A	Sed	18,613						2.0	0.0
132	8/23/2008	C81A	Sed	3,459						10.0	0.0
133	8/25/2008	C81A	Sed	393,382						0.0	1.1
134	8/26/2008	C81A	Sed	430,874						0.0	1.4
135	8/29/2008	C81A	Sed	694,836						1.0	2.3
136	9/26/2008	C81A	Sed	261,549						0.0	0.5
137	7/8/2008	C81B	Sed	35,940						0.0	0.6
138	7/11/2008	C81B	Sed	10,110						3.0	0.0
139	8/13/2008	C81B	Sed	93,969						0.0	0.6
140	8/25/2008	C81B	Sed	298,298						0.0	1.1

	Date	Site	Sample Location	*E. coli* Density (MPN/100ml)	TSS (mg/l)	Cond (µS/cm)	DO (mg/l)	pH	Temp (°C)	DSLR (days)	Rainfall (in)
141	8/26/2008	C81B	Sed	462,305						0.0	1.4
142	5/13/2008	CC	Sed	10,251						1.0	0.0
143	5/15/2008	CC	Sed	29,976						3.0	0.0
144	5/16/2008	CC	Sed	26,883						0.5	0.4
145	5/17/2008	CC	Sed	6,322						1.5	0.0
146	5/18/2008	CC	Sed	2,045,314						2.5	0.0
147	5/19/2008	CC	Sed	706,574						0.5	0.1
148	5/23/2008	CC	Sed	781						4.5	0.0
149	5/30/2008	CC	Sed	42,326						11.5	0.0
150	6/6/2008	CC	Sed	35,078						18.5	0.0
151	6/13/2008	CC	Sed	40,635						18.5	0.0
152	6/20/2008	CC	Sed	7,004						25.5	0.0
153	6/27/2008	CC	Sed	452						25.5	0.0
154	7/4/2008	CC	Sed	1,236						32.5	0.0
155	7/8/2008	CC	Sed	2,357						0.0	1.0
156	7/11/2008	CC	Sed	7,728						3.0	0.0
157	7/19/2008	CC	Sed	3,859						10.0	0.0
158	7/22/2008	CC	Sed	35,201						0.5	0.7
159	7/25/2008	CC	Sed	17,891						3.5	0.0
160	7/27/2008	CC	Sed	11,287						0.5	0.2
161	7/30/2008	CC	Sed	2,203						3.5	0.0
162	8/1/2008	CC	Sed	3,313						5.5	0.0
163	8/8/2008	CC	Sed	1,503						12.5	0.0
164	8/13/2008	CC	Sed	46,473						0.0	0.8
165	8/15/2008	CC	Sed	43,751						2.0	0.0
166	8/23/2008	CC	Sed	12,310						10.0	0.0
167	8/25/2008	CC	Sed	33,824						0.0	0.6
168	8/26/2008	CC	Sed	49,670						0.0	1.1
169	8/29/2008	CC	Sed	68,375						1.0	2.6
170	8/13/2008	CH1	Sed	535,913						0.0	0.7
171	8/15/2008	CH1	Sed	1,182,185						2.0	0.0
172	8/25/2008	CH1	Sed	79,280						0.0	0.6
173	8/26/2008	CH1	Sed	683,516						0.0	1.6
174	8/29/2008	CH1	Sed	411,080						1.0	2.4
175	9/26/2008	CH1	Sed	41,564						0.0	0.6
176	8/13/2008	CH2	Sed	393,588						0.0	0.7
177	8/15/2008	CH2	Sed	205,589						2.0	0.0
178	8/25/2008	CH2	Sed	72,394						0.0	0.6
179	8/26/2008	CH2	Sed	576,282						0.0	1.6
180	8/29/2008	CH2	Sed	1,083,385						1.0	2.4
181	9/26/2008	CH2	Sed	12,691						0.0	0.6
182	7/8/2008	RP	Sed	36,960						0.0	0.3
183	7/11/2008	RP	Sed	938,469						3.0	0.0
184	7/22/2008	RP	Sed	73,631						0.5	1.0
185	7/25/2008	RP	Sed	107,642						3.5	0.0
186	7/27/2008	RP	Sed	239,376						0.5	0.7
187	7/30/2008	RP	Sed	32,540						3.5	0.0
188	8/1/2008	RP	Sed	35,634						5.5	0.0
189	8/13/2008	RP	Sed	2,440						0.0	0.6
190	8/15/2008	RP	Sed	15,716						2.0	0.0

	Date	Site	Sample Location	*E. coli* Density (MPN/100ml)	TSS (mg/l)	Cond (µS/cm)	DO (mg/l)	pH	Temp (°C)	DSLR (days)	Rainfall (in)
191	8/25/2008	RP	Sed	28,363						0.0	0.8
192	8/26/2008	RP	Sed	1,143,389						0.0	1.6
193	8/29/2008	RP	Sed	886,296						1.0	2.7
194	5/30/2008	SC	Sed	15,787						11.5	0.0
195	7/8/2008	SC	Sed	221,099						0.0	0.4
196	7/22/2008	SC	Sed	374,259						0.5	0.7
197	7/27/2008	SC	Sed	10,255						0.5	0.7
198	8/13/2008	SC	Sed	36,421						0.0	0.8
199	8/15/2008	SC	Sed	154,589						2.0	0.0
200	8/25/2008	SC	Sed	215,494						0.0	1.0
201	8/26/2008	SC	Sed	35,446						0.0	1.5
202	8/29/2008	SC	Sed	15,885						1.0	2.4
203	7/22/2008	AHC	Outlet	43	64.0					0.5	1.0
204	7/27/2008	AHC	Outlet	15	101.8					0.5	0.7
205	8/13/2008	AHC	Outlet	25	256.6					0.0	0.4
206	8/25/2008	AHC	Outlet	2,420	330.1					0.0	0.7
207	8/26/2008	AHC	Outlet	2,420	185.0					0.0	1.6
208	8/29/2008	AHC	Outlet	56	23.0					1.0	2.4
209	9/26/2008	AHC	Outlet	1,414	98.3					0.0	0.6
210	7/27/2008	C81A	Outlet	435	51.0					0.5	0.7
211	8/13/2008	C81A	Outlet	1,203	122.8					0.0	0.6
212	8/25/2008	C81A	Outlet	2,420	369.4					0.0	1.1
213	8/26/2008	C81A	Outlet	1,733	179.9					0.0	1.4
214	8/29/2008	C81A	Outlet	2,420	15.7					1.0	2.3
215	7/8/2008	C81B	Outlet	2,420	29.7					0.0	0.6
216	8/13/2008	C81B	Outlet	816	48.4					0.0	0.6
217	8/25/2008	C81B	Outlet	1,553	11.4					0.0	1.1
218	8/26/2008	C81B	Outlet	1,414	45.4					0.0	1.4
219	5/13/2008	CC	Outlet	11	49.5					1.0	0.0
220	5/15/2008	CC	Outlet	13	10.3					3.0	0.0
221	5/16/2008	CC	Outlet	84	17.8					0.5	0.4
222	5/17/2008	CC	Outlet	16	9.3					1.5	0.0
223	5/18/2008	CC	Outlet	6	6.9					2.5	0.0
224	5/19/2008	CC	Outlet	15	6.5					0.5	0.1
225	5/23/2008	CC	Outlet	17	6.1					4.5	0.0
226	5/30/2008	CC	Outlet	23	6.2					11.5	0.0
227	6/6/2008	CC	Outlet	73	4.8					18.5	0.0
228	6/13/2008	CC	Outlet	99	8.6					18.5	0.0
229	6/20/2008	CC	Outlet	17	8.2					25.5	0.0
230	6/27/2008	CC	Outlet	33	6.0					25.5	0.0
231	7/4/2008	CC	Outlet	71	11.7					32.5	0.0
232	7/8/2008	CC	Outlet	816	151.4					0.0	1.0
233	7/11/2008	CC	Outlet	29	33.5					0.0	0.0
234	7/19/2008	CC	Outlet	46	19.9					10.0	0.0
235	7/22/2008	CC	Outlet	579	99.6					0.5	0.7
236	7/25/2008	CC	Outlet	102	10.9					3.5	0.0
237	7/27/2008	CC	Outlet	204	16.9					0.5	0.2
238	7/30/2008	CC	Outlet	39	14.4					3.5	0.0
239	8/1/2008	CC	Outlet	111	14.0					5.5	0.0
240	8/8/2008	CC	Outlet	37	19.1					12.5	0.0

	Date	Site	Sample Location	*E. coli* Density (MPN/100ml)	TSS (mg/l)	Cond (µS/cm)	DO (mg/l)	pH	Temp (℃)	DSLR (days)	Rainfall (in)
241	8/13/2008	CC	Outlet	1,300	33.1					0.0	0.8
242	8/15/2008	CC	Outlet	387	52.2					2.0	0.0
243	8/23/2008	CC	Outlet	8	14.1					10.0	0.0
244	8/25/2008	CC	Outlet	980	28.4					0.0	0.6
245	8/26/2008	CC	Outlet	2,420	48.4					0.0	1.1
246	8/29/2008	CC	Outlet	86	44.0					1.0	2.6
247	8/26/2008	CH1	Outlet	2,420	227.0					0.0	1.6
248	9/26/2008	CH1	Outlet	770	79.6					0.0	0.6
249	8/26/2008	CH2	Outlet	2,420	67.2					0.0	1.6
250	9/26/2008	CH2	Outlet	649	97.1					0.0	0.6
251	7/8/2008	RP	Outlet	1,300	260.4					0.0	0.3
252	7/22/2008	RP	Outlet	1,733	99.0					0.5	1.0
253	8/13/2008	RP	Outlet	770	47.7					0.0	0.6
254	8/25/2008	RP	Outlet	2,420	98.1					0.0	0.8
255	8/26/2008	RP	Outlet	2,420	123.0					0.0	1.6
256	7/27/2008	SC	Outlet	330	56.5					0.5	0.7
257	8/13/2008	SC	Outlet	2,420	130.5					0.0	0.8
258	8/25/2008	SC	Outlet	435	133.1					0.0	1.0
259	8/26/2008	SC	Outlet	770	142.3					0.0	1.5
260	7/27/2008	AHC	Inlet	72	522.3					0.5	0.7
261	8/13/2008	AHC	Inlet	17	359.0					0.0	0.4
262	8/25/2008	AHC	Inlet	102	304.3					0.0	0.7
263	8/26/2008	AHC	Inlet	1,733	168.4					0.0	1.6
264	9/26/2008	AHC	Inlet	649	307.5					0.0	0.6
265	8/25/2008	C81B	Inlet	50	16.9					0.0	1.1
266	8/26/2008	C81B	Inlet	178	18.5					0.0	1.4
267	8/26/2008	CH1	Inlet	285	3.8					0.0	1.6
268	9/26/2008	CH1	Inlet	292	158.5					0.0	0.6
269	8/26/2008	CH2	Inlet	1,986	65.2					0.0	1.6
270	9/26/2008	CH2	Inlet	259	320.8					0.0	0.6
271	8/13/2008	RP	Inlet	326	41.8					0.0	0.6
272	8/25/2008	RP	Inlet	2,420	23.6					0.0	0.8
273	8/26/2008	RP	Inlet	2,420	14.9					0.0	1.6